PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

Date of mailing (day/month/year) 22 May 2001 (22.05.01)	
International application No. PCT/EP00/07835	**Applicant's or agent's file reference** K 2840 Wd
International filing date (day/month/year) 11 August 2000 (11.08.00)	**Priority date** (day/month/year) 13 August 1999 (13.08.99)
Applicant NÜESCH, Jürg et al	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   10 March 2001 (10.03.01)

   [ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Claudio Borton

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 February 2001 (22.02.2001)

PCT

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WO 01/12666 A1

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99115161.4 13 August 1999 (13.08.1999) EP

(71) Applicant *(for all designated States except US)*: DEUTSCHES KREBSFORSCHUNGSZENTRUM [DE/DE]; Stiftung des öffentlichen Rechts, Im Neuenheimer Feld 280, D-69120 Heidelberg (DE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: NÜESCH, Jürg [CH/CH]; In den Wegscheiden 1, CH-4132 Muttenz (CH). ROMMELAERE, Jean [BE/DE]; Schloss Wolfsbrunnenweg 11, D-69118 Heidelberg (DE).

(74) Agent: SCHÜSSLER, Andrea; Huber & Schüssler, Truderinger Strasse 246, D-81825 München (DE).

(81) Designated States *(national)*: JP, US.

Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*
— *With (an) indication(s) in relation to deposited biological material furnished under Rule 13bis separately from the description.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/12666 A1

(54) Title: PARVOVIRUS NS1 VARIANTS

(57) Abstract: The present invention relates to a parvovirus NS1 variant having a shifted equilibrium between the DNA replication and transcription activities (a) and the cytotoxicity activity (b). Furthermore, this invention relates to DNAs coding for these parvovirus NS1 variants and methods of producing them. Additionally, this invention concerns antibodies directed against the parvovirus NS1 variants as well as the use of the DNAs and the parvovirus NS1 variants.

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* K 2840 Wd

Box No. I TITLE OF INVENTION

Parvovirus NS1 Variants

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

Deutsches Krebsforschungszentrum
Stiftung des öffentlichen Rechts
Im Neuenheimer Feld 280
69120 Heidelberg

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(that is, country)* of nationality: DE

State *(that is, country)* of residence: DE

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

Nüesch, Jürg
In den Wegscheiden 1
CH-4132 Muttenz

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: CH

State *(that is, country)* of residence: CH

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Dr. Andrea Schüßler
HUBER & SCHÜSSLER
Patentanwälte · Patent Attorneys
Truderinger Straße 246 · 81825 München
Tel. 089/42 72 47 48 · Fax 089/42 72 47 49

Telephone No.

Facsimile No.

Teleprinter No.

☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Sheet No. 2

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: (*Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.*)

Rommelaere, Jean
Schloß Wolfsbrunnenweg 11
D-69118 Heidelberg

This person is:
- [ ] applicant only
- [x] applicant and inventor
- [ ] inventor only (*If this check-box is marked, do not fill in below.*)

State (*that is, country*) of nationality: B

State (*that is, country*) of residence: DE

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [x] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: (*Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.*)

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only (*If this check-box is marked, do not fill in below.*)

State (*that is, country*) of nationality:

State (*that is, country*) of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: (*Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.*)

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only (*If this check-box is marked, do not fill in below.*)

State (*that is, country*) of nationality:

State (*that is, country*) of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: (*Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.*)

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only (*If this check-box is marked, do not fill in below.*)

State (*that is, country*) of nationality:

State (*that is, country*) of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

[ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

Sheet No. 3

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

☐ **AP** **ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, MZ Mozambique, SD Sudan, SL Sierra Leone, SZ Swaziland, TZ United Republic of Tanzania, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT

☐ **EA** **Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

☐ **EP** **European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☐ **OA** **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

☐ **AE** United Arab Emirates
☐ **AG** Antigua and Barbuda
☐ **AL** Albania .....
☐ **AM** Armenia .....
☐ **AT** Austria .....
☐ **AU** Australia .....
☐ **AZ** Azerbaijan
☐ **BA** Bosnia and Herzegovina .....
☐ **BB** Barbados
☐ **BG** Bulgaria .....
☐ **BR** Brazil .....
☐ **BY** Belarus .....
☐ **BZ** Belize
☐ **CA** Canada
☐ **CH and LI** Switzerland and Liechtenstein
☐ **CN** China .....
☐ **CR** Costa Rica .....
☐ **CU** Cuba .....
☐ **CZ** Czech Republic .....
☐ **DE** Germany .....
☐ **DK** Denmark .....
☐ **DM** Dominica
☐ **DZ** Algeria .....
☐ **EE** Estonia .....
☐ **ES** Spain .....
☐ **FI** Finland .....
☐ **GB** United Kingdom
☐ **GD** Grenada
☐ **GE** Georgia .....
☐ **GH** Ghana .....
☐ **GM** Gambia
☐ **HR** Croatia .....
☐ **HU** Hungary .....
☐ **ID** Indonesia
☐ **IL** Israel .....
☐ **IN** India .....
☐ **IS** Iceland
☒ **JP** Japan .....
☐ **KE** Kenya .....
☐ **KG** Kyrgyzstan .....
☐ **KP** Democratic People's Republic of Korea .....
☐ **KR** Republic of Korea .....
☐ **KZ** Kazakhstan .....
☐ **LC** Saint Lucia
☐ **LK** Sri Lanka
☐ **LR** Liberia
☐ **LS** Lesotho .....
☐ **LT** Lithuania
☐ **LU** Luxembourg
☐ **LV** Latvia
☐ **MA** Morocco .....
☐ **MD** Republic of Moldova .....
☐ **MG** Madagascar .....
☐ **MK** The former Yugoslav Republic of Macedonia .....
☐ **MN** Mongolia
☐ **MW** Malawi .....
☐ **MX** Mexico .....
☐ **MZ** Mozambique
☐ **NO** Norway
☐ **NZ** New Zealand .....
☐ **PL** Poland .....
☐ **PT** Portugal .....
☐ **RO** Romania
☐ **RU** Russian Federation .....
☐ **SD** Sudan
☐ **SE** Sweden
☐ **SG** Singapore
☐ **SI** Slovenia .....
☐ **SK** Slovakia .....
☐ **SL** Sierra Leone .....
☐ **TJ** Tajikistan .....
☐ **TM** Turkmenistan .....
☐ **TR** Turkey .....
☐ **TT** Trinidad and Tobago .....
☐ **TZ** United Republic of Tanzania
☐ **UA** Ukraine .....
☐ **UG** Uganda .....
☒ **US** United States of America .....
☐ **UZ** Uzbekistan .....
☐ **VN** Viet Nam .....
☐ **YU** Yugoslavia .....
☐ **ZA** South Africa .....
☐ **ZW** Zimbabwe .....

Check-box reserved for designating States which have become party to the PCT after issuance of this sheet:

☐ .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation (including fees) must reach the receiving Office within the 15-month time limit.)*

Sheet No. 4

Box No. VI PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) Aug. 13, 1999	99 115 161.4		EPO	
item (2)				
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): 1

* *Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):* ISA / EP

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*
Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets**:

request	:	4
description (excluding sequence listing part)	:	10
claims	:	2
abstract	:	1
drawings	:	6
sequence listing part of description	:	27
Total number of sheets	:	50

This international application is **accompanied** by the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☒ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☒ separate indications concerning deposited microorganism or other biological material
8. ☒ nucleotide and/or amino acid sequence listing in computer readable form
9. ☐ other *(specify)*:

Figure of the drawings which should accompany the abstract:

Language of filing of the international application:

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

A. Schüßler

Dr. Andrea Schüßler
European Patent Attorney

München, 10. Aug. 2000

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA /
6. ☐ Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
K 2840 Wd		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 00/ 07835	11/08/2000	13/08/1999

Applicant

DEUTSCHES KREBSFORSCHUNGSZENTRUM et al.

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[X] contained in the international application in written form.

[X] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 00/07835

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 14 and 15 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the NS1 variant.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTE TIONAL SEARCH REPORT

International Application No
PCT/EP 00/07835

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/35 C07K14/015 C07K16/08 G01N33/569 C12Q1/70
A61K35/76 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, STRAND, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	D LEGENDRE & J ROMMELAERE: "Terminal regions of the NS-1 protein of the parvovirus Minute Virus of Mice are involved in cytotoxicity and promoter trans inhibition" JOURNAL OF VIROLOGY, vol. 66, no. 10, October 1992 (1992-10), pages 5705-5713, XP000867510 AMERICAN SOCIETY FOR MICROBIOLOGY US *mutants pMMBal31 and pULB3201; figure 1 and page 5710 first paragraph* --- -/--	1-5,7-11

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 January 2001	30. 01. 01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Cupido, M

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/07835

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LI X ET AL: "Mutation of lysine 405 to serine in the parvovirus H-1 NS1 abolishes its function for viral DNA replication, late promoter activation, and cytotoxocity" JOURNAL OF VIROLOGY, vol. 64, no. 10, October 1990 (1990-10), pages 4654-4660, XP000867496 AMERICAN SOCIETY FOR MICROBIOLOGY US page 4656 -page 4657	1,3,7, 9-11
A	J P F NÜESCH ET AL: "Sequence motifs in the replicator protein of parvovirus MVM essential for nicking and covalent attachement to the viral origin: identification of the linking tyrosine" VIROLOGY,US,ACADEMIC PRESS,ORLANDO, vol. 209, no. 1, 10 May 1995 (1995-05-10), pages 122-135, XP002088311 ISSN: 0042-6822 page 127 -page 131	1-13
A	S F COTTMORE ET AL: "The NS1 polypeptide of the murine parvovirus MVM binds to DNA sequences containing the motif (ACCA)2-3" JOURNAL OF VIROLOGY,US,THE AMERICAN SOCIETY FOR MICROBIOLOGY, vol. 69, no. 3, pages 1652-1660, XP002088309 ISSN: 0022-538X page 1658, left-hand column, last paragraph -right-hand column	1-13

1

The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/_____

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty.

For International Preliminary Examining Authority use only	
Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION

Applicant's or agent's file reference: K 2840 Wd

International application No.	International filing date *(day/month/year)*	(Earliest) Priority date *(day/month/year)*
PCT/EP00/07835	Aug. 11, 2000	Aug. 13, 1999

Title of invention: Parvovirus NS1 Variants

Box No. II APPLICANT(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Deutsches Krebsforschungszentrum
Stiftung des öffentlichen Rechts
Im Neuenheimer Feld 280
69120 Heidelberg

Telephone No.:

Facsimile No.:

Teleprinter No.:

State *(i.e. country)* of nationality:	State *(i.e. country)* of residence:
DE	DE

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

NÜESCH, Jürg
In den Wegscheiden 1
CH-4132 Muttenz

State *(i.e. country)* of nationality:	State *(i.e. country)* of residence:
CH	CH

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

ROMMELAERE, Jean
Schloß Wolfsbrunnenweg 11
D-69118 Heidelberg

State *(i.e. country)* of nationality:	State *(i.e. country)* of residence:
DE	DE

☐ Further applicants are indicated on a continuation sheet.

Sheet No. .2.

International application No.
PCT/EP00/07835

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is [x] agent [ ] common representative

and [x] has been appointed earlier and represents the applicant(s) also for international preliminary examination.

[ ] is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

[ ] is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Dr. Andrea Schüßler

HUBER & SCHÜSSLER
Patentanwälte · Patent Attorneys
Truderinger Straße 246 · 81825 München
Tel. 089/42 72 47 48 · Fax 089/42 72 47 49

Telephone No.:

Facsimile No.:

Teleprinter No.:

[ ] Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV STATEMENT CONCERNING AMENDMENTS

The applicant wishes the International Preliminary Examining Authority*

(i) [ ] to start the international preliminary examination on the basis of the international application as originally filed.

(ii) [ ] to take into account the amendments under Article 34 of

- [ ] the description (amendments attached).
- [ ] the claims (amendments attached).
- [ ] the drawings (amendments attached).

(iii) [ ] to take into account any amendments of the claims under Article 19 filed with the International Bureau (a copy is attached).

(iv) [ ] to disregard any amendments of the claims made under Article 19 and to consider them as reversed.

(v) [ ] to postpone the start of the international preliminary examination until the expiration of 20 months from the priority date unless that Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Box No. V ELECTION OF STATES

[X] The applicant hereby elects all eligible States *(that is, all States which have been designated and which are bound by Chapter II of the PCT)* except .....

(If the applicant does not wish to elect certain eligible States, the name(s) or country code(s) of those States must be indicated above.)

Sheet No. 3.

International application No.
PCT/EP00/07835

Box No. VI CHECK LIST

The demand is accompanied by the following documents for the purposes of international preliminary examination:			For International Preliminary Examining Authority use only: received	not received
1. amendments under Article 34				
description	:	sheets	☐	☐
claims	:	sheets	☐	☐
drawings	:	sheets	☐	☐
2. letter accompanying amendments under Article 34	:	sheets	☐	☐
3. copy of amendments under Article 19	:	sheets	☐	☐
4. copy of statement under Article 19	:	sheets	☐	☐
5. other *(specify)*:	:	sheets	☐	☐

The demand is also accompanied by the item(s) marked below:

1. ☐ separate signed power of attorney
2. ☐ copy of general power of attorney
3. ☐ statement explaining lack of signature
4. ☒ fee calculation sheet
5. ☒ other *(specify)*: cheque

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

March 9, 2001

A. Schüßler

Dr. Andrea Schüßler
European Patent Attorney

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):
3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. ☐ The applicant has been informed accordingly.
4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.
5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

SCHÜSSLER, Andrea
HUBER & SCHÜSSLER
Truderinger Strasse 246
D-81825 München
ALLEMAGNE

Huber & Schüßler
Patentanwälte
0 3. DEZ. 2001
Frist: .....

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	29.11.2001
Applicant's or agent's file reference K 2840 Wd	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/07835	11/08/2000	13/08/1999

Applicant
DEUTSCHES KREBSFORSCHUNGSZENTRUM et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Cardenas, C Tel.+31 70 340-3370

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
K 2840 Wd		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/07835	11/08/2000	13/08/1999
International Patent Classification (IPC) or national classification and IPC C07K14/015		
Applicant DEUTSCHES KREBSFORSCHUNGSZENTRUM et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the International application

Date of submission of the demand	Date of completion of this report
10/03/2001	29.11.2001
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized officer Cupido, M Telephone No. +31 70 340 3374

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/07835

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-10	as originally filed				
Claims, No.:					
14,15	as originally filed				
1-13	as received on	06/11/2001	with letter of	06/11/2001	
Drawings, sheets:					
1/6-6/6	as originally filed				

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/07835

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under Item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 12,13.

because:

   ☒ the said international application, or the said claims Nos. 12,13 relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/07835

1. Statement

Novelty (N)	Yes:	Claims	4, 10, 11
	No:	Claims	1-3, 5-9
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-11
Industrial applicability (IA)	Yes:	Claims	1-11
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/EP00/07835

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 12 and 13 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

For the assessment of these claims on the question whether they are industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but will allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

I Document

The following document has been taken into consideration:

D1: J. Virol. 66, 5705-5713 (Legendre and Rommelaere, 1992)

II Novelty

D1 discloses a number of mutants in the NS1 protein of the parvovirus MVM. According to figure 1, mutants pMMBa131 and pULB3201 are still of cytotoxicity class I, showing that cytotoxicity is maintained although at a reduced level, but still regarded as being highly toxic, see page 5709 last paragraph, whereas P38 transactivation and DNA replication are strongly inhibited. pMMBa131 contains a deletion of amino acids 245-313, pULB3201 produces an NS1 with a deletion of 374 amino acids (from positions

167 to 547), and these mutations are therefore located at the positions referred to in claim 5. Consequently, there can be no doubt that D1 describes NS-1 mutants and their nucleic acids with the characteristics of the NS-1 mutants and DNAs claimed in claims 1-3 and 5 -9, and these claims lack novelty in view of Article 33(2) PCT.

III Inventive step

1. D1 is regarded as the closest prior art with respect to the question whether the claimed subject-matter involves an inventive step. The problem underlying the present application in view of D1 is the provision of further parvovirus NS1 proteins that can be used as a toxin for treating tumoral diseases.

2. The novel solutions to this problem provided and claimed in claim 4 of the present invention consist of four specific NS1 variants, designated as S283A, T363A, T394A and T463A. According to Table 1, these NS1 variants with the exception of T463A, are still cytotoxic, whereas P38 transactivation and DNA replication are strongly reduced. These variants can be regarded as possible candidates for use as a toxin in antitumour treatments. Hence, the subject-matter regarding NS1 variants, S283A, T363A and T394A is regarded to involve an inventive step as required by Article 33(3) PCT. Subject-matter relating to the non-toxic NS1 variant T463A is regarded not to involve an inventive step.

3. The antibody and kit referred to in claims 10 and 11 are characterised by the protein sequences to which these antibodies are directed. Since these antigen sequences are known from D1, and antibodies directed to known antigens are devoid of an inventive step, claims 10 and 11 also do not involve an inventive step and these claims cannot be accepted in view of Article 33(3) PCT.

Re Item VIII

Certain observations on the international application

Claim 6 refers to DNA coding for parvovirus NS1 variants having the following phosphorylation site mutants: S283A, T363A, T394A, or T463A, wherein the DNA comprises the DNA of figure 1. The DNA of figure 1 represents the wild-type NS1. Hence, the claim is contradictory and violates the requirements of Article 6 PCT.

K 2840

Claims

1. A parvovirus NS1 variant having a shifted equilibrium between the DNA replication and transcription activities (a) and the cytotoxicity activity (b), wherein

   - the activities (a) are reduced and eliminated, respectively, and activity (b) is maintained or increased or

   - activity (b) is reduced and eliminated, respectively, and the activities (a) are maintained or increased.

2. The parvovirus NS1 variant according to claim 1, wherein one or several phosphorylation sites are mutated.

3. The parvovirus NS1 variant according to claim 2, wherein the mutations are located at sites 283, 363, 394 and/or 463.

4. The parvovirus NS1 variant according to claim 2 or 3, namely the NS1 variants S283A, T363A, T394A, and T463A.

5. A DNA, coding for the parvovirus NS1 variant according to any one of claims 1 to 4.

6. The DNA according to claim 5, wherein the DNA comprises:

   (a) the DNA of figure 1,
   (b) a DNA hybridizing with the DNA from (a), said DNA comprising the mutated phosphorylation site of the DNA from (a), or
   (c) a DNA related to the DNA from (a) or (b) via the degenerated genetic code.

7. An expression vector, comprising the DNA according to

claim 5 or 6.

8. A transformant, containing the expression vector according to claim 7.

9. A method of producing the parvovirus NS1 variant according to any one of claims 1 to 4, comprising the culturing of the transformant according to claim 8 under suitable conditions.

10. An antibody, directed against the parvovirus NS1 variant according to any one of claims 1 to 4.

11. Kit comprising:

(a) a parvovirus NS1 variant according to the invention,

(b) a DNA according to the invention, e.g. an expression vector, particularly a parvovirus,

(c) an antibody according to the invention, as well as

(d) conventional auxiliary agents, such as solvents, buffers, carriers, markers and controls,

wherein of components (a) to (d) one or more representatives can be present each.

12. Use of the parvovirus NS1 variant according to any one of claims 1 to 4 as a toxin for treating tumoral diseases.

13. Use of the DNA according to claim 7 as a vector for gene therapy.

Replaced by Article 34

Claims

1. A parvovirus NS1 variant having a shifted equilibrium between the DNA replication and transcription activities (a) and the cytotoxicity activity (b).

2. The parvovirus NS1 variant according to claim 1, wherein the activities (a) are reduced and eliminated, respectively, and activity (b) is maintained or increased.

3. The parvovirus NS1 variant according to claim 1, wherein activity (b) is reduced and eliminated, respectively, and the activities (a) are maintained or increased.

4. The parvovirus NS1 variant according to any one of claims 1 to 3, wherein one or several phosphorylation sites are mutated.

5. The parvovirus NS1 variant according to claim 4, wherein the mutations are located at sites 283, 363, 394 and/or 463.

6. The parvovirus NS1 variant according to claim 4 or 5, namely the NS1 variants S283A, T363A, T394A, and T463A.

7. A DNA, coding for the parvovirus NS1 variant according to any one of claims 1 to 6.

8. The DNA according to claim 7, wherein the DNA comprises:

   (a) the DNA of figure 1,
   (b) a DNA hybridizing with the DNA from (a), said DNA comprising the mutated phosphorylation site of the DNA from (a), or
   (c) a DNA related to the DNA from (a) or (b) via the degenerated genetic code.

9. An expression vector, comprising the DNA according to claim 7 or 8.

10. A transformant, containing the expression vector according to claim 9.

11. A method of producing the parvovirus NS1 variant according to any one of claims 1 to 6, comprising the culturing of the transformant according to claim 10 under suitable conditions.

12. An antibody, directed against the parvovirus NS1 variant according to any one of claims 1 to 6.

13. Kit comprising:
(a) a parvovirus NS1 variant according to the invention,
(b) a DNA according to the invention, e.g. an expression vector, particularly a parvovirus,
(c) an antibody according to the invention, as well as
(d) conventional auxiliary agents, such as solvents, buffers, carriers, markers and controls,

wherein of components (a) to (d) one or more representatives can be present each.

14. Use of the parvovirus NS1 variant according to any one of claims 1 to 6 as a toxin for treating tumoral diseases.

15. Use of the DNA according to claim 9 as a vector for gene therapy.

Summary

The present invention relates to a parvovirus NS1 variant having a shifted equilibrium between the DNA replication and transcription activities (a) and the cytotoxicity activity (b). Furthermore, this invention relates to DNAs coding for these parvovirus NS1 variants and methods of producing them. Additionally, this invention concerns antibodies directed against the parvovirus NS1 variants as well as the use of the DNAs and the parvovirus NS1 variants.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

2

Applicant's or agent's file reference	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
K 2840 Wd	**FOR FURTHER ACTION**	
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/07835	11/08/2000	13/08/1999
International Patent Classification (IPC) or national classification and IPC C07K14/015		
Applicant DEUTSCHES KREBSFORSCHUNGSZENTRUM et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
10/03/2001	29.11.2001
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized officer Cupido, M Telephone No. +31 70 340 3374

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/07835

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, pages:

1-10	as originally filed				

Claims, No.:

14,15	as originally filed				
1-13	as received on	06/11/2001	with letter of	06/11/2001	

Drawings, sheets:

1/6-6/6	as originally filed				

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 12,13.

because:

   ☒ the said international application, or the said claims Nos. 12,13 relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	4, 10, 11
	No:	Claims	1-3, 5-9
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-11
Industrial applicability (IA)	Yes:	Claims	1-11
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 12 and 13 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

For the assessment of these claims on the question whether they are industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but will allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

I Document

The following document has been taken into consideration:

D1: J. Virol. **66**, 5705-5713 (Legendre and Rommelaere, 1992)

II Novelty

D1 discloses a number of mutants in the NS1 protein of the parvovirus MVM. According to figure 1, mutants pMMBa131 and pULB3201 are still of cytotoxicity class I, showing that cytotoxicity is maintained although at a reduced level, but still regarded as being highly toxic, see page 5709 last paragraph, whereas P38 transactivation and DNA replication are strongly inhibited. pMMBa131 contains a deletion of amino acids 245-313, pULB3201 produces an NS1 with a deletion of 374 amino acids (from positions

167 to 547), and these mutations are therefore located at the positions referred to in claim 5. Consequently, there can be no doubt that D1 describes NS-1 mutants and their nucleic acids with the characteristics of the NS-1 mutants and DNAs claimed in claims 1-3 and 5 -9, and these claims lack novelty in view of Article 33(2) PCT.

III Inventive step

1. D1 is regarded as the closest prior art with respect to the question whether the claimed subject-matter involves an inventive step. The problem underlying the present application in view of D1 is the provision of further parvovirus NS1 proteins that can be used as a toxin for treating tumoral diseases.

2. The novel solutions to this problem provided and claimed in claim 4 of the present invention consist of four specific NS1 variants, designated as S283A, T363A, T394A and T463A. According to Table 1, these NS1 variants with the exception of T463A, are still cytotoxic, whereas P38 transactivation and DNA replication are strongly reduced. These variants can be regarded as possible candidates for use as a toxin in antitumour treatments. Hence, the subject-matter regarding NS1 variants, S283A, T363A and T394A is regarded to involve an inventive step as required by Article 33(3) PCT. Subject-matter relating to the non-toxic NS1 variant T463A is regarded not to involve an inventive step.

3. The antibody and kit referred to in claims 10 and 11 are characterised by the protein sequences to which these antibodies are directed. Since these antigen sequences are known from D1, and antibodies directed to known antigens are devoid of an inventive step, claims 10 and 11 also do not involve an inventive step and these claims cannot be accepted in view of Article 33(3) PCT.

Re Item VIII

Certain observations on the international application

Claim 6 refers to DNA coding for parvovirus NS1 variants having the following phosphorylation site mutants: S283A, T363A, T394A, or T463A, wherein the DNA comprises the DNA of figure 1. The DNA of figure 1 represents the wild-type NS1. Hence, the claim is contradictory and violates the requirements of Article 6 PCT.

USA

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
K 2840 Wd		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 00/ 07835	11/08/2000	13/08/1999
Applicant		
DEUTSCHES KREBSFORSCHUNGSZENTRUM et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[X] contained in the international application in written form.

[X] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 00/07835

Box I Observations wher certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in r spect of certain claims under Article 17(2)(a) for th following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

~~Although claims 14 and 15 are directed to a method of treatment of the~~ human/animal body, the search has been carried out and based on the alleged effects of the NS1 variant.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

B x II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] Th additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/07835

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/35 C07K14/015 C07K16/08 G01N33/569 C12Q1/70
A61K35/76 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, STRAND, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	D LEGENDRE & J ROMMELAERE: "Terminal regions of the NS-1 protein of the parvovirus Minute Virus of Mice are involved in cytotoxicity and promoter trans inhibition" JOURNAL OF VIROLOGY, vol. 66, no. 10, October 1992 (1992-10), pages 5705-5713, XP000867510 AMERICAN SOCIETY FOR MICROBIOLOGY US *mutants pMMBal31 and pULB3201; figure 1 and page 5710 first paragraph* --- -/--	1-5,7-11

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 January 2001	30. 01. 01
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Cupido, M

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LI X ET AL: "Mutation of lysine 405 to serine in the parvovirus H-1 NS1 abolishes its function for viral DNA replication, late promoter activation, and cytotoxocity" JOURNAL OF VIROLOGY, vol. 64, no. 10, October 1990 (1990-10), pages 4654-4660, XP000867496 AMERICAN SOCIETY FOR MICROBIOLOGY US page 4656 -page 4657 ---	1,3,7, 9-11
A	J P F NÜESCH ET AL: "Sequence motifs in the replicator protein of parvovirus MVM essential for nicking and covalent attachement to the viral origin: identification of the linking tyrosine" VIROLOGY,US,ACADEMIC PRESS,ORLANDO, vol. 209, no. 1, 10 May 1995 (1995-05-10), pages 122-135, XP002088311 ISSN: 0042-6822 page 127 -page 131 ---	1-13
A	S F COTTMORE ET AL: "The NS1 polypeptide of the murine parvovirus MVM binds to DNA sequences containing the motif (ACCA)2-3" JOURNAL OF VIROLOGY,US,THE AMERICAN SOCIETY FOR MICROBIOLOGY, vol. 69, no. 3, pages 1652-1660, XP002088309 ISSN: 0022-538X page 1658, left-hand column, last paragraph -right-hand column -----	1-13

1

Parvovirus NS1 Variants

The present invention relates to parvovirus NS1 variants, DNAs coding for them and methods of producing the parvovirus NS1 variants. Furthermore, this invention concerns antibodies directed against the parvovirus NS1 variants as well as the use of the DNAs and the parvovirus NS1 variants.

Parvovirus designates a genus of the virus family Parvoviridae. The parvovirus genus comprises a number of small, icosaedric viruses that can replicate in the absence of a helper virus. Parvovirus contains a single-stranded DNA having a length of about 5.000 bp. At the 3' and 5' ends of the DNA there is one palindromic sequence each. The DNA codes for two capsid proteins, VP1 and VP2, as well as for two regulatory non-structure proteins, NS-1 and NS-2. The latter proteins are phosphorylated and show nuclear or both cytoplasmic and nuclear localization, respectively. NS1 is necessary for viral DNA replication and participates in the regulation of viral gene expression. Particularly, NS1 transactivates the promoter P38 and exhibits DNA-binding, helicase and DNA-nicking activities. Furthermore, NS1 induces cytotoxic and/or cytostatic stress in sensitive host cells.

Parvoviruses are usually well-tolerated by populations of their natural host, in which they persist without apparent pathological signs. This is due to both the protection of foetuses and neonates by maternal immunity, and the striking restriction of parvovirus replication to a narrow range of target proliferating tissues in adult animals. This host tolerance concerns especially rodent parvoviruses, for example the minute virus of mice (MVM) and H-1 virus in their respective natural hosts, namely mice and rats. In addition, humans can be infected with the latter viruses, without any evidence of associated deleterious effects from existing

epidemiological studies and clinical trials. On the other hand, it is known that certain parvoviruses, and especially rodent parvoviruses, are both oncotropic, i.e. accumulate preferentially in neoplastic versus normal tissues, and oncosuppressive, i.e. have a tumor-suppressive effect towards tumor cells, in various animal models. At least part of the oncosuppressive effect is thought to be due to a direct oncolytic action mediated by NS1. This oncosuppressive effect was also demonstrated against human tumor cells transplanted in recipient animals.

It is considered to use parvoviruses for therapeutic purposes. On the one hand, it seems to be of interest to use parvoviruses as vectors for therapeutic genes, i.e. for introducing such genes into the genome of cells. On the other hand, it is considered to use NS1 of parvoviruses as a toxin for treating tumoral diseases. However, initial experiments showed unsatisfactory results.

Therefore, it is the object of the present invention to provide a product by which parvoviruses and NS1 thereof, respectively, can be used for the above purposes.

According to the invention this is achieved by the subject matters defined in the claims.

The present invention is based on the applicant's findings that it is possible to interfere with the activities of parvovirus NS1 so as to shift the equilibrium existing between the DNA replication and transcription activities (a) and the cytotoxicity activity (b). In particular, he produced parvovirus NS1 variants in which the DNA replication and transcription activities (a) are reduced and eliminated, respectively, whereas the cytotoxicity activity (b) is maintained or raised. Moreover, he produced parvovirus NS1 variants in which the cytotoxicity activity (b) is reduced and eliminated, respectively, whereas the DNA replication and transcription activities (a) are maintained or raised.

Examples of such parvovirus NS1 variants are indicated in Table 1 and figure 1. In addition, the applicant recognized that the above parvovirus NS1 variants and expression vectors coding for them, particularly parvoviruses, respectively, are suitable for therapeutic purposes.

According to the invention, the applicant's findings are used to provide a parvovirus NS1 variant in which the equilibrium between the DNA replication and transcription activities (a) and the cytotoxicity activity (b) is shifted.

The expression "parvovirus" comprises any parvovirus, particularly a rodent parvovirus, such as minute virus of mice (MVM) and H-1 virus.

The expression "the equilibrium between the DNA replication and transcription activities (a) and the cytotoxicity activity (b) is shifted" refers to the fact that in a parvovirus NS1 variant according to the invention such an equilibrium is shifted as compared to the parvovirus NS1 wild-type. In particular, the equilibrium can be shifted to the effect that the DNA replication and transcription activities (a) are reduced and eliminated, respectively, whereas the cytotoxicity activity (b) is maintained or raised. The cytotoxicity activity (b) can also be reduced and eliminated, respectively, whereas the DNA replication and transcription activities (a) are maintained or raised. Such an equilibrium can be determined by various methods. As regards the determination of the DNA replication activity, reference is made e.g. to methods described in Legendre and Rommelaere, 1992, J. Virol. 66, 5705; Cotmore et al., 1992, Virology 190, 365; Cotmore et al., 1993, J. Virol. 67, 1579, Cotmore and Tattersall, 1994, Embo. J. 13, 4145. As to the determination of the transcription activity reference is made to methods described e.g. in Rhode and Richards, 1987, J. Virol. 61, 2807. Regarding the determination of the cytotoxicity activity reference is made to the below examples.

According to the invention parvovirus NS1 variants are preferred in which the shift of equilibrium is achieved by mutation of one or several phosphorylation sites. Particularly preferred are parvovirus NS1 variants which have a mutation at one or several of the phosphorylation sites 283, 363, 394 and 463. Even more preferred are the parvovirus NS1 variants S283A, T363A, T394A and T463A, which are indicated in Table 1 and figure 1. In S283A, a serine is exchanged by an alanine at position 283, in T363A a threonine is exchanged by alanine at position 363, in T394A a threonine is exchanged by alanine at position 394 and in T 463A a threonine is exchanged by alanine at position 463.

A further subject matter of the present invention relates to a nucleic acid, particularly a DNA, which codes for an above parvovirus NS1 variant. Such a DNA comprises preferably:

(a) the DNA of fig. 1.1, 1.2, 1.3 and 1.4, respectively
(b) a DNA hybridizing with the DNA from (a), said DNA comprising the mutated phosphorylation site of the DNA from (a), or
(c) a DNA related to the DNA from (a) or (b) via the degenerated genetic code.

The DNA of (a) was deposited with DSMZ (Deutsche Sammlung von Mikroorganismen and Zellkulturen) on Aug. 11, 1999, i.e. fig. 1.1 as Escherichia coli pRSV-NS:S283A under DSM 12994, fig. 1.2 as Escherichia coli pRSV-NS:T363A under DSM 12995, fig. 1.3. as Escherichia coli pRSV-NS:T394A under DSM 12996 and fig. 1.4 as Escherichia coli pRSV-NS:T463A under DSM 12997.

The expression "hybridizing DNA" refers to a DNA which hybridizes with a DNA from (a) under normal conditions, particularly at 20(C below the melting point of the DNA. In this connection, the expression "hybridizing" refers to conventional hybridization conditions, preferably to hybridization conditions where 5xSSPE, 1 % SDS, 1xDenhardt's solution are used as solution and the hybridization

temperatures are between 35(C and 70(C, preferably 65(C. The hybridization is followed by a wash step first carried out with 2xSSC, 1 % SDS and then with 0.2xSSC at temperatures between 35(C and 70(C, preferably at 65(C. Furthermore, reference is made to Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory Press, cold Spring Harbor NY (1989).

A DNA according to the invention can be present in a vector and expression vector, respectively. A person skilled in the art is familiar with examples thereof. In the case of an expression vector for E. coli these are e.g. pGEMEX, pUC derivatives, pGEX-2T, pET3b, T7 based expression vectors and pQE-8. For the expression in yeast, e.g. pY100 and Ycpad1 have to be mentioned while e.g. pKCR, pEFBOS, cDM8, pMSCND, and pCEV4 have to be indicated for the expression in animal cells. The baculovirus expression vector pAcSGHisNT-A is especially suitable for the expression in insect cells.

In a preferred embodiment, the vector containing the DNA according to the invention is a virus, e.g. an adenovirus, vaccinia virus, an AAV virus or a parvovirus, such as MVM or H-1, a parvovirus being preferred. The vector may also be a retrovirus, such as MoMULV, MoMuLV, HaMuSV, MuMTV, RSV or GaLV.

For constructing expression vectors which contain the DNA according to the invention, it is possible to use general methods known in the art. These methods include e.g. in vitro recombination techniques, synthetic methods and in vivo recombination methods as described in Sambrook et al., supra, for example.

Furthermore, the present invention relates to host cells which contain the above described vectors. These host cells include bacteria, yeast, insect and animal cells, preferably mammalian cells. The E. coli strains HB101, DH1, x1776, JM101, JM109, BL21, XL1Blue and SG 13009, the yeast strain

Saccharomyces cerevisiae and the animal cells L, A9, 3T3, FM3A, CHO, COS, Vero, HeLa and the insect cells sf9 are preferred. Methods of transforming these host cells, of phenotypically selecting transformants and of expressing the DNA according to the invention by using the above described vectors are known in the art.

Moreover, the present invention relates to antibodies which specifically recognize an above describe parvovirus NS1 variant, i.e. the region of the parvovirus NS1 variant where the mutation responsible for the shifted equilibrium, particularly a mutated phosphorylation site, is located. The antibodies can be monoclonal, polyclonal or synthetic antibodies or fragments thereof, e.g. Fab, Fv or scFV fragments. Preferably monoclonal antibodies are concerned. For the production it is favorable to immunize animals - particularly rabbits or chickens for a polyclonal antibody and mice for a monoclonal antibody - with an above parvovirus NS1 variant or with fragments thereof. Further boosters of the animals can be effected with the same parvovirus NS1 variant or with fragments thereof. The polyclonal antibody can then be obtained from the animal serum and egg yolk, respectively. The monoclonal antibody can be obtained according to standard methods, reference being made particularly to the method by K÷hler and Milstein (Nature 256 (1975), 495) and GalfrÚ (Meth. Enzymol. 73 (1981), 3). In this case, mouse myeloma cells are fused with spleen cells originating from the immunized animals. Antibodies according to the invention can be used in many ways, e.g. for the immunoprecipitation of the above described parvovirus NS1 variants or for the isolation thereof. The antibodies can be bound in immunoassays in liquid phase or to a solid carrier. In this connection, the antibodies can be labeled in various ways. The person skilled in the art is familiar with suitable markers and labeling methods. Examples of immunoassays are ELISA and RIA.

The present invention provides parvovirus NS1 variants in which the equilibrium between the DNA replication and

transcription activities (a) and the cytotoxicity activity (b) is shifted. In particular, parvovirus NS1 variants are provided which have a reduced or no cytotoxicity activity, whereas the DNA replication and transcription activities are maintained or increased. Parvovirus NS1 variants are also provided in which the DNA replication and transcription activities are reduced and eliminated, respectively, whereas the cytotoxicity activity is maintained or raised. Thus, the present invention provides products which are suitable for therapeutic purposes. In particular, expression vectors according to the invention, e.g. parvoviruses, can be used for gene-therapeutic measures. Moreover, parvoviruses NS1 variants according to the invention are suitable as toxins, e.g. for treating tumoral diseases.

Therefore, a kit is also provided for the application of the present invention. This kit comprises the following:

(a) a parvovirus NS1 variant according to the invention,
(b) a DNA according to the invention, e.g. an expression vector, particularly a parvovirus,
(c) an antibody according to the invention, as well as
(d) conventional auxiliary agents, such as solvents, buffers, carriers, markers and controls.

Of component (a) to (d) one or more representatives can be present each.

Brief description of the drawings

Fig. 1 shows the DNA and amino acid sequences of parvovirus NS1 variants according to the invention (fig. 1.1, 1.2, 1.3 and 1.4) as compared to a parvovirus NS1 wild-type. In this connection, the mutated sites in the parvovirus NS1 variants according to the invention are labeled each.

The present invention is explained by the examples.

Example 1: Preparation and purification of NS1 variants according to the invention

The DNA of the NS1 variant S283A according to the invention was provided as an EcoRV to BstEII fragment obtained by chimeric PCR using two mutagenic primers. This fragment was then inserted into the corresponding cleaved expression vector pTHisNS1 (Nuesch et al., Virology 209, (1995), 122) to obtain pTHis NS1:S283A. Such a vector codes for a fusion protein comprising 6 histidine residues (N terminus partner) and S283A of Fig. 1 (C terminus partner). For expression and purification of S283A the NS1 gene under control of the bacteriophage T7 promoter was transferred into vaccinia virus and expressed in eucaryotic cells by double infection together with vTF7-3 (a vaccinia virus expressing the bacteriophage T7 DNA polymerase). 18 hrs post infection cells were harvested and nuclear extracts prepared. The histidine tagged S283A was then purified by affinity chromatography on Ni-NTA agarose and analyzed by 10 % SDS-PAGE (Nuesch et al., supra).

It showed that a parvovirus NS1 variant according to the invention can be prepared in highly pure form.

The NS1 variants T363A, T394A, and T463A were also produced and purified in the same way.

Example 2: Preparation and detection of an antibody according to the invention

Tubes were coated with purified NS1 variants prepared as in example 1 and monoclonal antibodies (e.g. scFv) specifically binding to S283A were isolated from human synthetic VH+VL scFV phage library (Griffith et al., EMBO J., 13, (1994), 3245) according to standard panning protocols after >5 isolation and amplification procedures. The variable region of the isolated scFv harbored in the phagemid were sequenced to identify NS1

variant interacting partner proteins harboring such binding motifs from comparison with known genes in the gene bank.

It showed that monoclonal antibodies according to the invention can be isolated.

In addition, the NS1 variants were used for immunization of animals in order to obtain poly- or monoclonal antibodies.

Example 3: Characterization of the parvovirus NS1 variants S283A, T363A, T394A and T463A according to the invention

The characterization of the parvovirus NS1 variants comprised the determination of the DNA replication, transcription, cytotoxicity, DNA binding, nicking and helicase activities. Known methods were used for this purpose (cf. description, supra). As regards the determination of the helicase activity reference is made to Stahl et al. 1986, EMBO J. 5, 1999. As to the determination of the nicking activity reference is made to Christensen et al., 1997, J. Virol. 71, 1405 and Nuesch et al., 1995, supra. Regarding the determination of the DNA binding reference is made to Cotmore et al. 1995, J. Virol. 69, 1652. As far as the determination of the cytotoxicity activity is concerned, the following steps were carried out: NS1 variants were transferred into an expression vector containing the NS1 gene under the control of the parvovirus MVMP4 promoter (genuine promoter driving the non-structural genes of MVM), and the green fluorescent protein (EGFP) under control of an additional promoter. These constructs were then transfected into A9 cells using lipofectamine (GibcoBRL) according to the manufacturer's instruction and the impact of the NS1 variant on the viability of the cells tested in time course experiments. Transfected cells were identified by fluorescence of the EGFP. Toxic effects were determined in comparison to wild type NS1 or a vector containing no NS1 gene as a function of time as well as a measure of cytopathic

changes on the cell morphology.

The data indicated in Table 1 were obtained:

Table 1

	S283A	T363A	T394A	T463A	wt
P38-TA	+	-	-	++++	++++
ACCA	+	++++	++	++	++
Nick-1	+	-	-	+++	+++
Nick-2	+++	-	-	++++	++++
Nick-3	++	-	-		++++
Heli	++	-	(+)	++++	++++
Rep	+	-	-	+	++++
Cyto	++++++	++	+++	(+)	+++

Example 4: NS1 variants' expression after transduction using recombinant viral vectors

NS1 expression cassettes containing the NS1 variants according to the invention under control of the parvoviral P4 promoter and a 3'untranslated region from parvovirus MVM to ensure stability and translation of the gene product, were transferred either in a parvovirus genome background as exemplified in example 3, or a heterologous viral genome background, such as vaccinia virus (example 1) or adenovirus. Promoter and terminator regions were exchanged according to the requirements. The nucleic acids containing the NS1 variants were then packaged either in vivo (after transient transfection into eucaryotic cells) or in vitro and the packaged transducing particles were isolated. These transducing units containing NS1 variants were used either for studies concerning gene regulation in tissue culture or animals, but also as therapeutic agents either alone or in combination with other agents (such as cytokines) in gene and cancer therapy approaches.

Claims

1. A parvovirus NS1 variant having a shifted equilibrium between the DNA replication and transcription activities (a) and the cytotoxicity activity (b).

2. The parvovirus NS1 variant according to claim 1, wherein the activities (a) are reduced and eliminated, respectively, and activity (b) is maintained or increased.

3. The parvovirus NS1 variant according to claim 1, wherein activity (b) is reduced and eliminated, respectively, and the activities (a) are maintained or increased.

4. The parvovirus NS1 variant according to any one of claims 1 to 3, wherein one or several phosphorylation sites are mutated.

5. The parvovirus NS1 variant according to claim 4, wherein the mutations are located at sites 283, 363, 394 and/or 463.

6. The parvovirus NS1 variant according to claim 4 or 5, namely the NS1 variants S283A, T363A, T394A, and T463A.

7. A DNA, coding for the parvovirus NS1 variant according to any one of claims 1 to 6.

8. The DNA according to claim 7, wherein the DNA comprises:

   (a) the DNA of figure 1,
   (b) a DNA hybridizing with the DNA from (a), said DNA comprising the mutated phosphorylation site of the DNA from (a), or
   (c) a DNA related to the DNA from (a) or (b) via the degenerated genetic code.

9. An expression vector, comprising the DNA according to claim 7 or 8.

10. A transformant, containing the expression vector according to claim 9.

11. A method of producing the parvovirus NS1 variant according to any one of claims 1 to 6, comprising the culturing of the transformant according to claim 10 under suitable conditions.

12. An antibody, directed against the parvovirus NS1 variant according to any one of claims 1 to 6.

13. Kit comprising:
(a) a parvovirus NS1 variant according to the invention,
(b) a DNA according to the invention, e.g. an expression vector, particularly a parvovirus,
(c) an antibody according to the invention, as well as
(d) conventional auxiliary agents, such as solvents, buffers, carriers, markers and controls,

wherein of components (a) to (d) one or more representatives can be present each.

14. Use of the parvovirus NS1 variant according to any one of claims 1 to 6 as a toxin for treating tumoral diseases.

15. Use of the DNA according to claim 9 as a vector for gene therapy.

Fig. 1

Wild-type NS1

```
    ATGGCTGGAAATGCTTACTCTGATGAAGTTTTGGGAGCAACCAACTGGTTAAAGGAAAAA
261 ---------+---------+---------+---------+---------+---------+ 320
    TACCGACCTTTACGAATGAGACTACTTCAAAACCCTCGTTGGTTGACCAATTTCCTTTTT

    M  A  G  N  A  Y  S  D  E  V  L  G  A  T  N  W  L  K  E  K  -

    AGTAACCAGGAAGTGTTCTCATTTGTTTTTAAAAATGAAAATGTTCAACTGAATGGAAAA
321 ---------+---------+---------+---------+---------+---------+ 380
    TCATTGGTCCTTCACAAGAGTAAACAAAAATTTTTACTTTTACAAGTTGACTTACCTTTT

    S  N  Q  E  V  F  S  F  V  F  K  N  E  N  V  Q  L  N  G  K  -

    GATATCGGATGGAATAGTTACAAAAAAGAGCTGCAGGAGGACGAGCTGAAATCTTTACAA
381 ---------+---------+---------+---------+---------+---------+ 440
    CTATAGCCTACCTTATCAATGTTTTTTCTCGACGTCCTCCTGCTCGACTTTAGAAATGTT

    D  I  G  W  N  S  Y  K  K  E  L  Q  E  D  E  L  K  S  L  Q  -

    CGAGGAGCGGAAACTACTTGGGACCAAAGCGAGGACATGGAATGGGAAACCACAGTGGAT
441 ---------+---------+---------+---------+---------+---------+ 500
    GCTCCTCGCCTTTGATGAACCCTGGTTTCGCTCCTGTACCTTACCCTTTGGTGTCACCTA

    R  G  A  E  T  T  W  D  Q  S  E  D  M  E  W  E  T  T  V  D  -

    GAAATGACCAAAAAGCAAGTATTCATTTTTGATTCTTTGGTTAAAAAATGTTTATTTGAA
501 ---------+---------+---------+---------+---------+---------+ 560
    CTTTACTGGTTTTTCGTTCATAAGTAAAAACTAAGAAACCAATTTTTTACAAATAAACTT

    E  M  T  K  K  Q  V  F  I  F  D  S  L  V  K  K  C  L  F  E  -

    GTGCTTAACACAAAGAATATATTTCCTGGTGATGTTAATTGGTTTGTGCAACATGAATGG
561 ---------+---------+---------+---------+---------+---------+ 620
    CACGAATTGTGTTTCTTATATAAAGGACCACTACAATTAACCAAACACGTTGTACTTACC

    V  L  N  T  K  N  I  F  P  G  D  V  N  W  F  V  Q  H  E  W  -

    GGAAAAGACCAAGGCTGGCACTGCCATGTACTAATTGGAGGAAAGGACTTTAGTCAAGCT
621 ---------+---------+---------+---------+---------+---------+ 680
    CCTTTTCTGGTTCCGACCGTGACGGTACATGATTAACCTCCTTTCCTGAAATCAGTTCGA

    G  K  D  Q  G  W  H  C  H  V  L  I  G  G  K  D  F  S  Q  A  -

    CAAGGGAAATGGTGGAGAAGGCAACTAAATGTTTACTGGAGCAGATGGTTGGTAACAGCC
681 ---------+---------+---------+---------+---------+---------+ 740
    GTTCCCTTTACCACCTCTTCCGTTGATTTACAAATGACCTCGTCTACCAACCATTGTCGG

    Q  G  K  W  W  R  R  Q  L  N  V  Y  W  S  R  W  L  V  T  A  -

    TGTAATGTGCAACTAACACCAGCTGAAAGAATTAAACTAAGAGAAATAGCAGAAGACAAT
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Fig. 1 (Fortsetzung I)

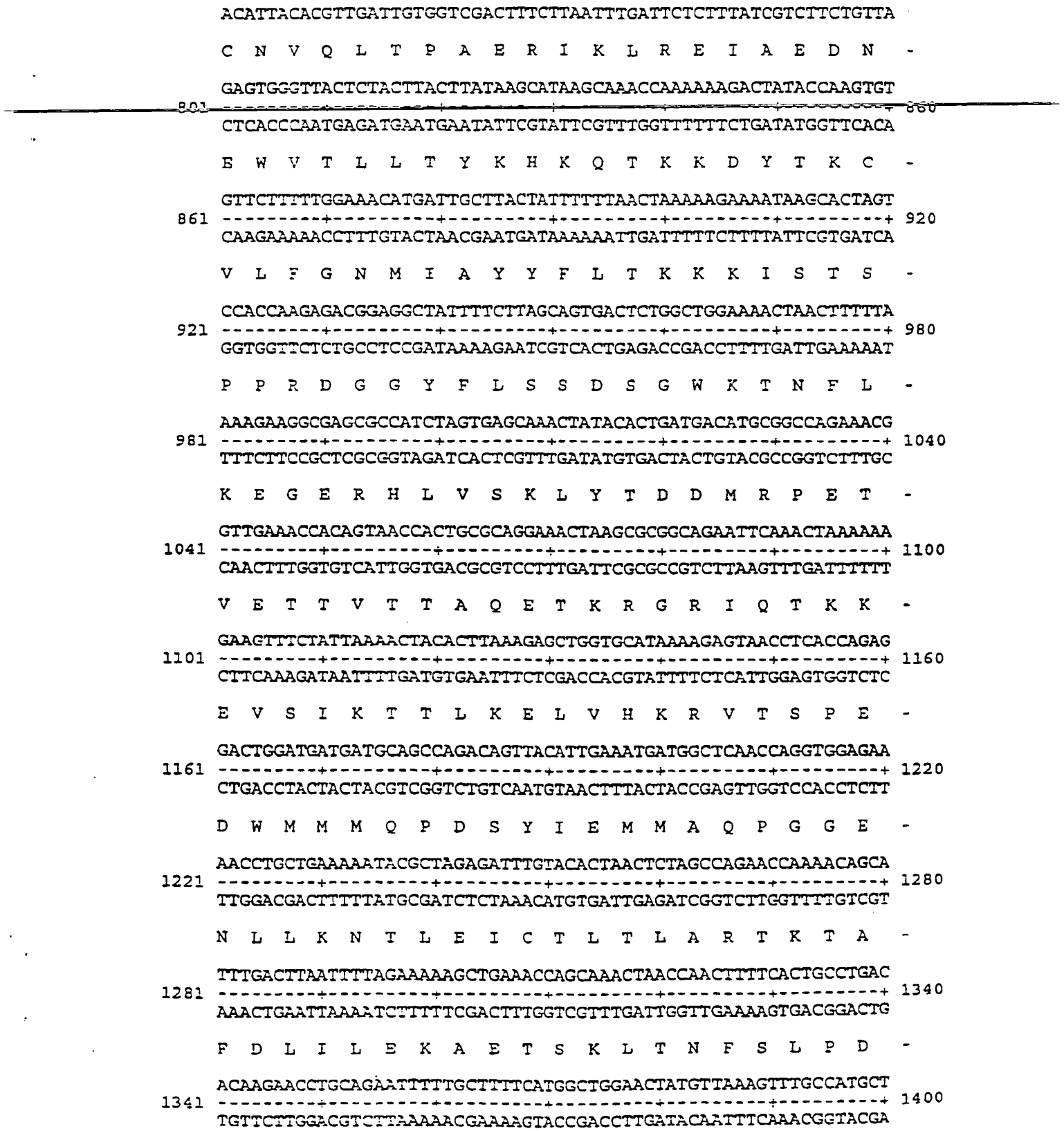

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Fig. 1 (Fortsetzung II)

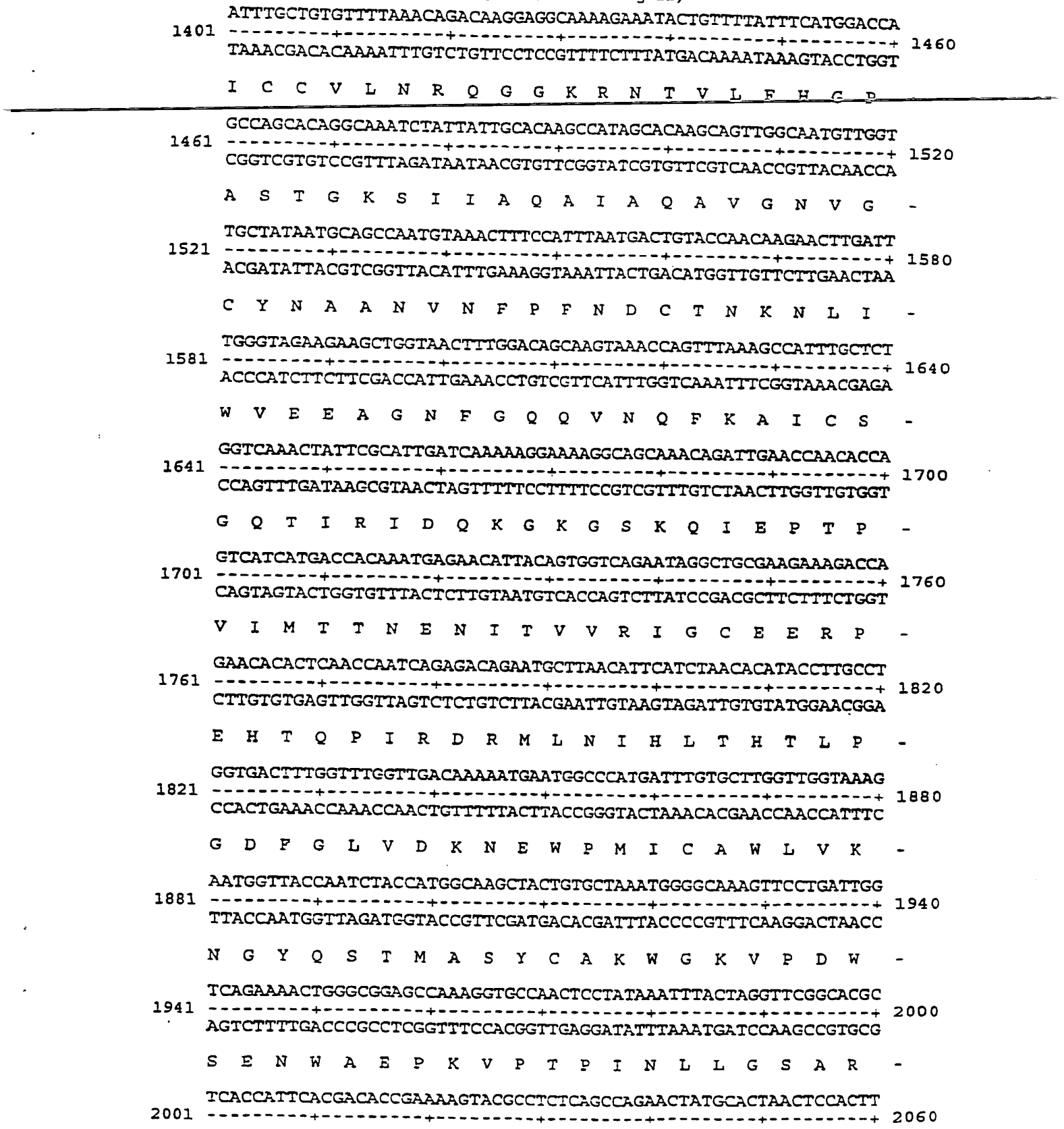

Fig. 1 (Fortsetzung III)

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2061 ---------+---------+---------+---------+---------+---------+ 2120
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     A  S  D  L  E  D  L  A  L  E  P  W  S  T  P  N  T  P  V  A  -

     GGCACTGCAGAAACCCAGAACACTGGGGAAGCTGGTTCCAAAGCCTGCCAAGATGGTCAA
2121 ---------+---------+---------+---------+---------+---------+ 2180
     CCGTGACGTCTTTGGGTCTTGTGACCCCTTCGACCAAGGTTTCGGACGGTTCTACCAGTT

     G  T  A  E  T  Q  N  T  G  E  A  G  S  K  A  C  Q  D  G  Q  -

     CTGAGCCCAACTTGGTCAGAGATCGAGGAGGATTTGAGAGCGTGCTTCGGTGCGGAACCG
2181 ---------+---------+---------+---------+---------+---------+ 2240
     GACTCGGGTTGAACCAGTCTCTAGCTCCTCCTAAACTCTCGCACGAAGCCACGCCTTGGC

     L  S  P  T  W  S  E  I  E  E  D  L  R  A  C  F  G  A  E  P  -

     TTGAAGAAAGACTTCAGCGAGCCGCTGAACTTGGACTAA
2241 ---------+---------+---------+--------- 2279
     AACTTCTTTCTGAAGTCGCTCGGCGACTTGAACCTGATT

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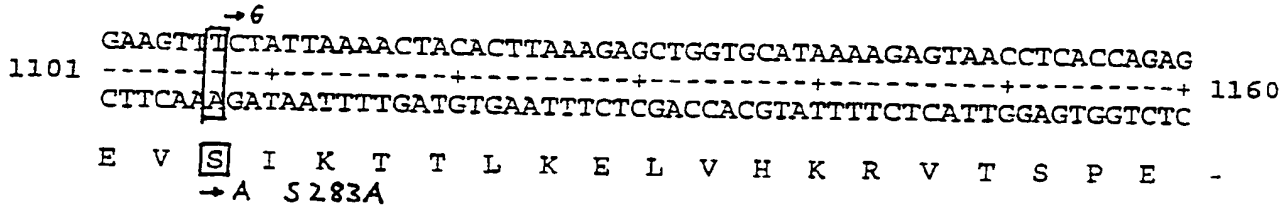
Fig. 1.1

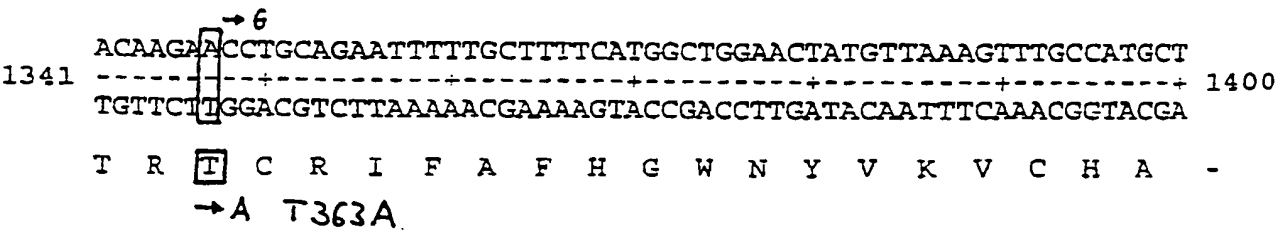
1100 - 261 Wildtype-NS1-Sequence



1161 - 2279 Wildtype-NS1-Sequence

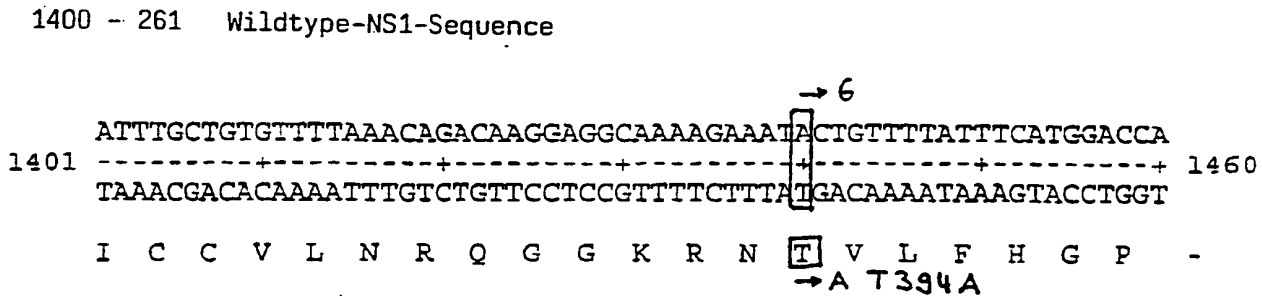
Fig. 1.2

1340 - 261 Wildtype-NS1-Sequence



1401 - 2279 Wildtype-NS1-Sequence

Fig. 1.3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1401 + + + + + + 1460
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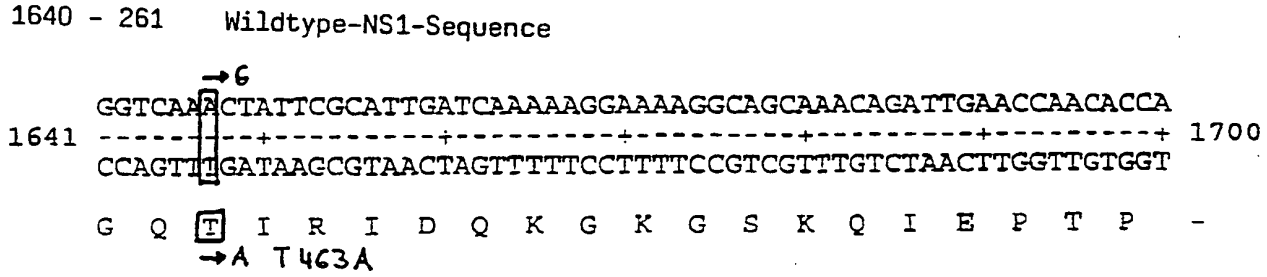
1461 - 2279 Wildtype-NS1-Sequence

Fig. 1.4


1640 - 261 Wildtype-NS1-Sequence
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1641 + + + + + + 1700
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→A T463A
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<110> Deutsches Krebsforschungszentrum

<120> Parvovirus NS1 Variants

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<151> 1999-08-13

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Leu Lys Glu Lys Ser Asn Gln Glu Val Phe Ser Phe Val Phe Lys Asn
            20                  25                  30

gaa aat gtt caa ctg aat gga aaa gat atc gga tgg aat agt tac aaa       144
Glu Asn Val Gln Leu Asn Gly Lys Asp Ile Gly Trp Asn Ser Tyr Lys
         35                  40                  45

aaa gag ctg cag gag gac gag ctg aaa tct tta caa cga gga gcg gaa       192
Lys Glu Leu Gln Glu Asp Glu Leu Lys Ser Leu Gln Arg Gly Ala Glu
     50                  55                  60

act act tgg gac caa agc gag gac atg gaa tgg gaa acc aca gtg gat       240
Thr Thr Trp Asp Gln Ser Glu Asp Met Glu Trp Glu Thr Thr Val Asp
 65                  70                  75                  80

gaa atg acc aaa aag caa gta ttc att ttt gat tct ttg gtt aaa aaa       288
Glu Met Thr Lys Lys Gln Val Phe Ile Phe Asp Ser Leu Val Lys Lys
                 85                  90                  95

tgt tta ttt gaa gtg ctt aac aca aag aat ata ttt cct ggt gat gtt       336
Cys Leu Phe Glu Val Leu Asn Thr Lys Asn Ile Phe Pro Gly Asp Val
            100                 105                 110

aat tgg ttt gtg caa cat gaa tgg gga aaa gac caa ggc tgg cac tgc       384
Asn Trp Phe Val Gln His Glu Trp Gly Lys Asp Gln Gly Trp His Cys
        115                 120                 125

cat gta cta att gga gga aag gac ttt agt caa gct caa ggg aaa tgg       432
His Val Leu Ile Gly Gly Lys Asp Phe Ser Gln Ala Gln Gly Lys Trp
    130                 135                 140
```

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145                 150                 155                 160

tgt aat gtg caa cta aca cca gct gaa aga att aaa cta aga gaa ata        528
Cys Asn Val Gln Leu Thr Pro Ala Glu Arg Ile Lys Leu Arg Glu Ile
                165                 170                 175

gca gaa gac aat gag tgg gtt act cta ctt act tat aag cat aag caa        576
Ala Glu Asp Asn Glu Trp Val Thr Leu Leu Thr Tyr Lys His Lys Gln
            180                 185                 190

acc aaa aaa gac tat acc aag tgt gtt ctt ttt gga aac atg att gct        624
Thr Lys Lys Asp Tyr Thr Lys Cys Val Leu Phe Gly Asn Met Ile Ala
        195                 200                 205

tac tat ttt tta act aaa aag aaa ata agc act agt cca cca aga gac        672
Tyr Tyr Phe Leu Thr Lys Lys Lys Ile Ser Thr Ser Pro Pro Arg Asp
    210                 215                 220

gga ggc tat ttt ctt agc agt gac tct ggc tgg aaa act aac ttt tta        720
Gly Gly Tyr Phe Leu Ser Ser Asp Ser Gly Trp Lys Thr Asn Phe Leu
225                 230                 235                 240

aaa gaa ggc gag cgc cat cta gtg agc aaa cta tac act gat gac atg        768
Lys Glu Gly Glu Arg His Leu Val Ser Lys Leu Tyr Thr Asp Asp Met
                245                 250                 255

cgg cca gaa acg gtt gaa acc aca gta acc act gcg cag gaa act aag        816
Arg Pro Glu Thr Val Glu Thr Thr Val Thr Thr Ala Gln Glu Thr Lys
            260                 265                 270

cgc ggc aga att caa act aaa aaa gaa gtt tct att aaa act aca ctt        864
Arg Gly Arg Ile Gln Thr Lys Lys Glu Val Ser Ile Lys Thr Thr Leu
        275                 280                 285

aaa gag ctg gtg cat aaa aga gta acc tca cca gag gac tgg atg atg        912
Lys Glu Leu Val His Lys Arg Val Thr Ser Pro Glu Asp Trp Met Met
    290                 295                 300

atg cag cca gac agt tac att gaa atg atg gct caa cca ggt gga gaa        960
Met Gln Pro Asp Ser Tyr Ile Glu Met Met Ala Gln Pro Gly Gly Glu
305                 310                 315                 320

aac ctg ctg aaa aat acg cta gag att tgt aca cta act cta gcc aga       1008
Asn Leu Leu Lys Asn Thr Leu Glu Ile Cys Thr Leu Thr Leu Ala Arg
                325                 330                 335

acc aaa aca gca ttt gac tta att tta gaa aaa gct gaa acc agc aaa       1056
Thr Lys Thr Ala Phe Asp Leu Ile Leu Glu Lys Ala Glu Thr Ser Lys
            340                 345                 350

cta acc aac ttt tca ctg cct gac aca aga acc tgc aga att ttt gct       1104
Leu Thr Asn Phe Ser Leu Pro Asp Thr Arg Thr Cys Arg Ile Phe Ala
        355                 360                 365

ttt cat ggc tgg aac tat gtt aaa gtt tgc cat gct att tgc tgt gtt       1152
Phe His Gly Trp Asn Tyr Val Lys Val Cys His Ala Ile Cys Cys Val
    370                 375                 380

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Leu Asn Arg Gln Gly Gly Lys Arg Asn Thr Val Leu Phe His Gly Pro
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gcc agc aca ggc aaa tct att att gca caa gcc ata gca caa gca gtt       1248
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gac tgt acc aac aag aac ttg att tgg gta gaa gaa gct ggt aac ttt      1344
Asp Cys Thr Asn Lys Asn Leu Ile Trp Val Glu Glu Ala Gly Asn Phe
        435                 440                 445

gga cag caa gta aac cag ttt aaa gcc att tgc tct ggt caa act att      1392
Gly Gln Gln Val Asn Gln Phe Lys Ala Ile Cys Ser Gly Gln Thr Ile
    450                 455                 460

cgc att gat caa aaa gga aaa ggc agc aaa cag att gaa cca aca cca      1440
Arg Ile Asp Gln Lys Gly Lys Gly Ser Lys Gln Ile Glu Pro Thr Pro
465                 470                 475                 480

gtc atc atg acc aca aat gag aac att aca gtg gtc aga ata ggc tgc      1488
Val Ile Met Thr Thr Asn Glu Asn Ile Thr Val Val Arg Ile Gly Cys
                485                 490                 495

gaa gaa aga cca gaa cac act caa cca atc aga gac aga atg ctt aac      1536
Glu Glu Arg Pro Glu His Thr Gln Pro Ile Arg Asp Arg Met Leu Asn
            500                 505                 510

att cat cta aca cat acc ttg cct ggt gac ttt ggt ttg gtt gac aaa      1584
Ile His Leu Thr His Thr Leu Pro Gly Asp Phe Gly Leu Val Asp Lys
        515                 520                 525

aat gaa tgg ccc atg att tgt gct tgg ttg gta aag aat ggt tac caa      1632
Asn Glu Trp Pro Met Ile Cys Ala Trp Leu Val Lys Asn Gly Tyr Gln
    530                 535                 540

tct acc atg gca agc tac tgt gct aaa tgg ggc aaa gtt cct gat tgg      1680
Ser Thr Met Ala Ser Tyr Cys Ala Lys Trp Gly Lys Val Pro Asp Trp
545                 550                 555                 560

tca gaa aac tgg gcg gag cca aag gtg cca act cct ata aat tta cta      1728
Ser Glu Asn Trp Ala Glu Pro Lys Val Pro Thr Pro Ile Asn Leu Leu
                565                 570                 575

ggt tcg gca cgc tca cca ttc acg aca ccg aaa agt acg cct ctc agc      1776
Gly Ser Ala Arg Ser Pro Phe Thr Thr Pro Lys Ser Thr Pro Leu Ser
            580                 585                 590

cag aac tat gca cta act cca ctt gca tcg gat ctc gag gac ctg gct      1824
Gln Asn Tyr Ala Leu Thr Pro Leu Ala Ser Asp Leu Glu Asp Leu Ala
        595                 600                 605

tta gag cct tgg agc aca cca aat act cct gtt gcg ggc act gca gaa      1872
Leu Glu Pro Trp Ser Thr Pro Asn Thr Pro Val Ala Gly Thr Ala Glu
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acc cag aac act ggg gaa gct ggt tcc aaa gcc tgc caa gat ggt caa      1920
Thr Gln Asn Thr Gly Glu Ala Gly Ser Lys Ala Cys Gln Asp Gly Gln
625                 630                 635                 640

ctg agc cca act tgg tca gag atc gag gag gat ttg aga gcg tgc ttc      1968
Leu Ser Pro Thr Trp Ser Glu Ile Glu Glu Asp Leu Arg Ala Cys Phe
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ggt gcg gaa ccg ttg aag aaa gac ttc agc gag ccg ctg aac ttg gac      2016
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Glu Asn Val Gln Leu Asn Gly Lys Asp Ile Gly Trp Asn Ser Tyr Lys
        35                  40                  45

Lys Glu Leu Gln Glu Asp Glu Leu Lys Ser Leu Gln Arg Gly Ala Glu
    50                  55                  60

Thr Thr Trp Asp Gln Ser Glu Asp Met Glu Trp Glu Thr Thr Val Asp
65                  70                  75                  80

Glu Met Thr Lys Lys Gln Val Phe Ile Phe Asp Ser Leu Val Lys Lys
                85                  90                  95

Cys Leu Phe Glu Val Leu Asn Thr Lys Asn Ile Phe Pro Gly Asp Val
            100                 105                 110

Asn Trp Phe Val Gln His Glu Trp Gly Lys Asp Gln Gly Trp His Cys
        115                 120                 125

His Val Leu Ile Gly Gly Lys Asp Phe Ser Gln Ala Gln Gly Lys Trp
    130                 135                 140

Trp Arg Arg Gln Leu Asn Val Tyr Trp Ser Arg Trp Leu Val Thr Ala
145                 150                 155                 160

Cys Asn Val Gln Leu Thr Pro Ala Glu Arg Ile Lys Leu Arg Glu Ile
                165                 170                 175

Ala Glu Asp Asn Glu Trp Val Thr Leu Leu Thr Tyr Lys His Lys Gln
            180                 185                 190

Thr Lys Lys Asp Tyr Thr Lys Cys Val Leu Phe Gly Asn Met Ile Ala
        195                 200                 205

Tyr Tyr Phe Leu Thr Lys Lys Lys Ile Ser Thr Ser Pro Pro Arg Asp
    210                 215                 220

Gly Gly Tyr Phe Leu Ser Ser Asp Ser Gly Trp Lys Thr Asn Phe Leu
225                 230                 235                 240

Lys Glu Gly Glu Arg His Leu Val Ser Lys Leu Tyr Thr Asp Asp Met
                245                 250                 255

Arg Pro Glu Thr Val Glu Thr Thr Val Thr Thr Ala Gln Glu Thr Lys
            260                 265                 270

Arg Gly Arg Ile Gln Thr Lys Lys Glu Val Ser Ile Lys Thr Thr Leu
        275                 280                 285

Lys Glu Leu Val His Lys Arg Val Thr Ser Pro Glu Asp Trp Met Met
    290                 295                 300

Met Gln Pro Asp Ser Tyr Ile Glu Met Met Ala Gln Pro Gly Gly Glu
305                 310                 315                 320
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Thr Lys Thr Ala Phe Asp Leu Ile Leu Glu Lys Ala Glu Thr Ser Lys
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Leu Thr Asn Phe Ser Leu Pro Asp Thr Arg Thr Cys Arg Ile Phe Ala
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Phe His Gly Trp Asn Tyr Val Lys Val Cys His Ala Ile Cys Cys Val
370 375 380

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385 390 395 400

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405 410 415

Gly Asn Val Gly Cys Tyr Asn Ala Ala Asn Val Asn Phe Pro Phe Asn
420 425 430

Asp Cys Thr Asn Lys Asn Leu Ile Trp Val Glu Glu Ala Gly Asn Phe
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Gly Gln Gln Val Asn Gln Phe Lys Ala Ile Cys Ser Gly Gln Thr Ile
450 455 460

Arg Ile Asp Gln Lys Gly Lys Gly Ser Lys Gln Ile Glu Pro Thr Pro
465 470 475 480

Val Ile Met Thr Thr Asn Glu Asn Ile Thr Val Val Arg Ile Gly Cys
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500 505 510

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515 520 525

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Ser Thr Met Ala Ser Tyr Cys Ala Lys Trp Gly Lys Val Pro Asp Trp
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Ser Glu Asn Trp Ala Glu Pro Lys Val Pro Thr Pro Ile Asn Leu Leu
565 570 575

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580 585 590

Gln Asn Tyr Ala Leu Thr Pro Leu Ala Ser Asp Leu Glu Asp Leu Ala
595 600 605

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1               5                   10                  15

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Leu Lys Glu Lys Ser Asn Gln Glu Val Phe Ser Phe Val Phe Lys Asn
            20                  25                  30

gaa aat gtt caa ctg aat gga aaa gat atc gga tgg aat agt tac aaa   144
Glu Asn Val Gln Leu Asn Gly Lys Asp Ile Gly Trp Asn Ser Tyr Lys
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Lys Glu Leu Gln Glu Asp Glu Leu Lys Ser Leu Gln Arg Gly Ala Glu
    50                  55                  60

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Thr Thr Trp Asp Gln Ser Glu Asp Met Glu Trp Glu Thr Thr Val Asp
65                  70                  75                  80

gaa atg acc aaa aag caa gta ttc att ttt gat tct ttg gtt aaa aaa   288
Glu Met Thr Lys Lys Gln Val Phe Ile Phe Asp Ser Leu Val Lys Lys
                85                  90                  95

tgt tta ttt gaa gtg ctt aac aca aag aat ata ttt cct ggt gat gtt   336
Cys Leu Phe Glu Val Leu Asn Thr Lys Asn Ile Phe Pro Gly Asp Val
            100                 105                 110
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aat tgg ttt gtg caa cat gaa tgg gga aaa gac caa ggc tgg cac tgc        384
Asn Trp Phe Val Gln His Glu Trp Gly Lys Asp Gln Gly Trp His Cys
        115                 120                 125

cat gta cta att gga gga aag gac ttt agt caa gct caa ggg aaa tgg        432
His Val Leu Ile Gly Gly Lys Asp Phe Ser Gln Ala Gln Gly Lys Trp
    130                 135                 140

tgg aga agg caa cta aat gtt tac tgg agc aga tgg ttg gta aca gcc        480
Trp Arg Arg Gln Leu Asn Val Tyr Trp Ser Arg Trp Leu Val Thr Ala
145                 150                 155                 160

tgt aat gtg caa cta aca cca gct gaa aga att aaa cta aga gaa ata        528
Cys Asn Val Gln Leu Thr Pro Ala Glu Arg Ile Lys Leu Arg Glu Ile
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gca gaa gac aat gag tgg gtt act cta ctt act tat aag cat aag caa        576
Ala Glu Asp Asn Glu Trp Val Thr Leu Leu Thr Tyr Lys His Lys Gln
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acc aaa aaa gac tat acc aag tgt gtt ctt ttt gga aac atg att gct        624
Thr Lys Lys Asp Tyr Thr Lys Cys Val Leu Phe Gly Asn Met Ile Ala
        195                 200                 205

tac tat ttt tta act aaa aag aaa ata agc act agt cca cca aga gac        672
Tyr Tyr Phe Leu Thr Lys Lys Lys Ile Ser Thr Ser Pro Pro Arg Asp
    210                 215                 220

gga ggc tat ttt ctt agc agt gac tct ggc tgg aaa act aac ttt tta        720
Gly Gly Tyr Phe Leu Ser Ser Asp Ser Gly Trp Lys Thr Asn Phe Leu
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aaa gaa ggc gag cgc cat cta gtg agc aaa cta tac act gat gac atg        768
Lys Glu Gly Glu Arg His Leu Val Ser Lys Leu Tyr Thr Asp Asp Met
                245                 250                 255

cgg cca gaa acg gtt gaa acc aca gta acc act gcg cag gaa act aag        816
Arg Pro Glu Thr Val Glu Thr Thr Val Thr Thr Ala Gln Glu Thr Lys
            260                 265                 270

cgc ggc aga att caa act aaa aaa gaa gtt gct att aaa act aca ctt        864
Arg Gly Arg Ile Gln Thr Lys Lys Glu Val Ala Ile Lys Thr Thr Leu
        275                 280                 285

aaa gag ctg gtg cat aaa aga gta acc tca cca gag gac tgg atg atg        912
Lys Glu Leu Val His Lys Arg Val Thr Ser Pro Glu Asp Trp Met Met
    290                 295                 300

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Met Gln Pro Asp Ser Tyr Ile Glu Met Met Ala Gln Pro Gly Gly Glu
305                 310                 315                 320

aac ctg ctg aaa aat acg cta gag att tgt aca cta act cta gcc aga       1008
Asn Leu Leu Lys Asn Thr Leu Glu Ile Cys Thr Leu Thr Leu Ala Arg
                325                 330                 335

acc aaa aca gca ttt gac tta att tta gaa aaa gct gaa acc agc aaa       1056
Thr Lys Thr Ala Phe Asp Leu Ile Leu Glu Lys Ala Glu Thr Ser Lys
            340                 345                 350

cta acc aac ttt tca ctg cct gac aca aga acc tgc aga att ttt gct       1104
Leu Thr Asn Phe Ser Leu Pro Asp Thr Arg Thr Cys Arg Ile Phe Ala
        355                 360                 365

ttt cat ggc tgg aac tat gtt aaa gtt tgc cat gct att tgc tgt gtt       1152
Phe His Gly Trp Asn Tyr Val Lys Val Cys His Ala Ile Cys Cys Val
    370                 375                 380
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tta aac aga caa gga ggc aaa aga aat act gtt tta ttt cat gga cca      1200
Leu Asn Arg Gln Gly Gly Lys Arg Asn Thr Val Leu Phe His Gly Pro
385                 390                 395                 400

gcc agc aca ggc aaa tct att att gca caa gcc ata gca caa gca gtt      1248
Ala Ser Thr Gly Lys Ser Ile Ile Ala Gln Ala Ile Ala Gln Ala Val
                405                 410                 415

ggc aat gtt ggt tgc tat aat gca gcc aat gta aac ttt cca ttt aat      1296
Gly Asn Val Gly Cys Tyr Asn Ala Ala Asn Val Asn Phe Pro Phe Asn
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gac tgt acc aac aag aac ttg att tgg gta gaa gaa gct ggt aac ttt      1344
Asp Cys Thr Asn Lys Asn Leu Ile Trp Val Glu Glu Ala Gly Asn Phe
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gga cag caa gta aac cag ttt aaa gcc att tgc tct ggt caa act att      1392
Gly Gln Gln Val Asn Gln Phe Lys Ala Ile Cys Ser Gly Gln Thr Ile
    450                 455                 460

cgc att gat caa aaa gga aaa ggc agc aaa cag att gaa cca aca cca      1440
Arg Ile Asp Gln Lys Gly Lys Gly Ser Lys Gln Ile Glu Pro Thr Pro
465                 470                 475                 480

gtc atc atg acc aca aat gag aac att aca gtg gtc aga ata ggc tgc      1488
Val Ile Met Thr Thr Asn Glu Asn Ile Thr Val Val Arg Ile Gly Cys
                485                 490                 495

gaa gaa aga cca gaa cac act caa cca atc aga gac aga atg ctt aac      1536
Glu Glu Arg Pro Glu His Thr Gln Pro Ile Arg Asp Arg Met Leu Asn
            500                 505                 510

att cat cta aca cat acc ttg cct ggt gac ttt ggt ttg gtt gac aaa      1584
Ile His Leu Thr His Thr Leu Pro Gly Asp Phe Gly Leu Val Asp Lys
        515                 520                 525

aat gaa tgg ccc atg att tgt gct tgg ttg gta aag aat ggt tac caa      1632
Asn Glu Trp Pro Met Ile Cys Ala Trp Leu Val Lys Asn Gly Tyr Gln
    530                 535                 540

tct acc atg gca agc tac tgt gct aaa tgg ggc aaa gtt cct gat tgg      1680
Ser Thr Met Ala Ser Tyr Cys Ala Lys Trp Gly Lys Val Pro Asp Trp
545                 550                 555                 560

tca gaa aac tgg gcg gag cca aag gtg cca act cct ata aat tta cta      1728
Ser Glu Asn Trp Ala Glu Pro Lys Val Pro Thr Pro Ile Asn Leu Leu
                565                 570                 575

ggt tcg gca cgc tca cca ttc acg aca ccg aaa agt acg cct ctc agc      1776
Gly Ser Ala Arg Ser Pro Phe Thr Thr Pro Lys Ser Thr Pro Leu Ser
            580                 585                 590

cag aac tat gca cta act cca ctt gca tcg gat ctc gag gac ctg gct      1824
Gln Asn Tyr Ala Leu Thr Pro Leu Ala Ser Asp Leu Glu Asp Leu Ala
        595                 600                 605

tta gag cct tgg agc aca cca aat act cct gtt gcg ggc act gca gaa      1872
Leu Glu Pro Trp Ser Thr Pro Asn Thr Pro Val Ala Gly Thr Ala Glu
    610                 615                 620

acc cag aac act ggg gaa gct ggt tcc aaa gcc tgc caa gat ggt caa      1920
Thr Gln Asn Thr Gly Glu Ala Gly Ser Lys Ala Cys Gln Asp Gly Gln
625                 630                 635                 640

ctg agc cca act tgg tca gag atc gag gag gat ttg aga gcg tgc ttc      1968
Leu Ser Pro Thr Trp Ser Glu Ile Glu Glu Asp Leu Arg Ala Cys Phe
                645                 650                 655
```

```
ggt gcg gaa ccg ttg aag aaa gac ttc agc gag ccg ctg aac ttg gac      2016
Gly Ala Glu Pro Leu Lys Lys Asp Phe Ser Glu Pro Leu Asn Leu Asp
            660                 665                 670

taa                                                                 2019
```

<210> 5
<211> 20
<212> PRT
<213> part of Parvovirus NS1 variant

<400> 5

```
Glu Val Ala Ile Lys Thr Thr Leu Lys Glu Leu Val His Lys Arg Val
1               5                   10                  15

Thr Ser Pro Glu
            20
```

<210> 6
<211> 672
<212> PRT
<213> Parvovirus NS1 variant

<400> 6

```
Met Ala Gly Asn Ala Tyr Ser Asp Glu Val Leu Gly Ala Thr Asn Trp
  1               5                   10                  15

Leu Lys Glu Lys Ser Asn Gln Glu Val Phe Ser Phe Val Phe Lys Asn
             20                  25                  30

Glu Asn Val Gln Leu Asn Gly Lys Asp Ile Gly Trp Asn Ser Tyr Lys
         35                  40                  45

Lys Glu Leu Gln Glu Asp Glu Leu Lys Ser Leu Gln Arg Gly Ala Glu
     50                  55                  60

Thr Thr Trp Asp Gln Ser Glu Asp Met Glu Trp Glu Thr Thr Val Asp
 65                  70                  75                  80

Glu Met Thr Lys Lys Gln Val Phe Ile Phe Asp Ser Leu Val Lys Lys
                 85                  90                  95

Cys Leu Phe Glu Val Leu Asn Thr Lys Asn Ile Phe Pro Gly Asp Val
            100                 105                 110

Asn Trp Phe Val Gln His Glu Trp Gly Lys Asp Gln Gly Trp His Cys
        115                 120                 125

His Val Leu Ile Gly Gly Lys Asp Phe Ser Gln Ala Gln Gly Lys Trp
    130                 135                 140

Trp Arg Arg Gln Leu Asn Val Tyr Trp Ser Arg Trp Leu Val Thr Ala
145                 150                 155                 160

Cys Asn Val Gln Leu Thr Pro Ala Glu Arg Ile Lys Leu Arg Glu Ile
                165                 170                 175

Ala Glu Asp Asn Glu Trp Val Thr Leu Leu Thr Tyr Lys His Lys Gln
            180                 185                 190
```

```
Thr Lys Lys Asp Tyr Thr Lys Cys Val Leu Phe Gly Asn Met Ile Ala
        195                 200                 205

Tyr Tyr Phe Leu Thr Lys Lys Lys Ile Ser Thr Ser Pro Pro Arg Asp
    210                 215                 220

Gly Gly Tyr Phe Leu Ser Ser Asp Ser Gly Trp Lys Thr Asn Phe Leu
225                 230                 235                 240

Lys Glu Gly Glu Arg His Leu Val Ser Lys Leu Tyr Thr Asp Asp Met
                245                 250                 255

Arg Pro Glu Thr Val Glu Thr Thr Val Thr Thr Ala Gln Glu Thr Lys
            260                 265                 270

Arg Gly Arg Ile Gln Thr Lys Lys Glu Val Ala Ile Lys Thr Thr Leu
        275                 280                 285

Lys Glu Leu Val His Lys Arg Val Thr Ser Pro Glu Asp Trp Met Met
    290                 295                 300

Met Gln Pro Asp Ser Tyr Ile Glu Met Met Ala Gln Pro Gly Gly Glu
305                 310                 315                 320

Asn Leu Leu Lys Asn Thr Leu Glu Ile Cys Thr Leu Thr Leu Ala Arg
                325                 330                 335

Thr Lys Thr Ala Phe Asp Leu Ile Leu Glu Lys Ala Glu Thr Ser Lys
            340                 345                 350

Leu Thr Asn Phe Ser Leu Pro Asp Thr Arg Thr Cys Arg Ile Phe Ala
        355                 360                 365

Phe His Gly Trp Asn Tyr Val Lys Val Cys His Ala Ile Cys Cys Val
    370                 375                 380

Leu Asn Arg Gln Gly Gly Lys Arg Asn Thr Val Leu Phe His Gly Pro
385                 390                 395                 400

Ala Ser Thr Gly Lys Ser Ile Ile Ala Gln Ala Ile Ala Gln Ala Val
                405                 410                 415

Gly Asn Val Gly Cys Tyr Asn Ala Ala Asn Val Asn Phe Pro Phe Asn
            420                 425                 430

Asp Cys Thr Asn Lys Asn Leu Ile Trp Val Glu Glu Ala Gly Asn Phe
        435                 440                 445

Gly Gln Gln Val Asn Gln Phe Lys Ala Ile Cys Ser Gly Gln Thr Ile
    450                 455                 460

Arg Ile Asp Gln Lys Gly Lys Gly Ser Lys Gln Ile Glu Pro Thr Pro
465                 470                 475                 480

Val Ile Met Thr Thr Asn Glu Asn Ile Thr Val Val Arg Ile Gly Cys
                485                 490                 495

Glu Glu Arg Pro Glu His Thr Gln Pro Ile Arg Asp Arg Met Leu Asn
            500                 505                 510

Ile His Leu Thr His Thr Leu Pro Gly Asp Phe Gly Leu Val Asp Lys
        515                 520                 525

Asn Glu Trp Pro Met Ile Cys Ala Trp Leu Val Lys Asn Gly Tyr Gln
    530                 535                 540
```

```
Ser Thr Met Ala Ser Tyr Cys Ala Lys Trp Gly Lys Val Pro Asp Trp
545                 550                 555                 560

Ser Glu Asn Trp Ala Glu Pro Lys Val Pro Thr Pro Ile Asn Leu Leu
                565                 570                 575

Gly Ser Ala Arg Ser Pro Phe Thr Thr Pro Lys Ser Thr Pro Leu Ser
            580                 585                 590

Gln Asn Tyr Ala Leu Thr Pro Leu Ala Ser Asp Leu Glu Asp Leu Ala
        595                 600                 605

Leu Glu Pro Trp Ser Thr Pro Asn Thr Pro Val Ala Gly Thr Ala Glu
    610                 615                 620

Thr Gln Asn Thr Gly Glu Ala Gly Ser Lys Ala Cys Gln Asp Gly Gln
625                 630                 635                 640

Leu Ser Pro Thr Trp Ser Glu Ile Glu Glu Asp Leu Arg Ala Cys Phe
                645                 650                 655

Gly Ala Glu Pro Leu Lys Lys Asp Phe Ser Glu Pro Leu Asn Leu Asp
            660                 665                 670
```

<210> 7
<211> 60
<212> DNA
<213> part of Parvovirus NS1 variant

<220>
<221> CDS
<222> (1)..(60)

<400> 7

```
aca aga gcc tgc aga att ttt gct ttt cat ggc tgg aac tat gtt aaa   48
Thr Arg Ala Cys Arg Ile Phe Ala Phe His Gly Trp Asn Tyr Val Lys
1               5                   10                  15

gtt tgc cat gct                                                  60
Val Cys His Ala
            20
```

<210> 8
<211> 2019
<212> DNA
<213> Parvovirus NS1 variant

<220>
<221> CDS
<222> (1)..(2016)

<400> 8

```
atg gct gga aat gct tac tct gat gaa gtt ttg gga gca acc aac tgg       48
Met Ala Gly Asn Ala Tyr Ser Asp Glu Val Leu Gly Ala Thr Asn Trp
1               5                   10                  15

tta aag gaa aaa agt aac cag gaa gtg ttc tca ttt gtt ttt aaa aat       96
Leu Lys Glu Lys Ser Asn Gln Glu Val Phe Ser Phe Val Phe Lys Asn
            20                  25                  30
```

```
gaa aat gtt caa ctg aat gga aaa gat atc gga tgg aat agt tac aaa       144
Glu Asn Val Gln Leu Asn Gly Lys Asp Ile Gly Trp Asn Ser Tyr Lys
        35                  40                  45

aaa gag ctg cag gag gac gag ctg aaa tct tta caa cga gga gcg gaa       192
Lys Glu Leu Gln Glu Asp Glu Leu Lys Ser Leu Gln Arg Gly Ala Glu
    50                  55                  60

act act tgg gac caa agc gag gac atg gaa tgg gaa acc aca gtg gat       240
Thr Thr Trp Asp Gln Ser Glu Asp Met Glu Trp Glu Thr Thr Val Asp
65                  70                  75                  80

gaa atg acc aaa aag caa gta ttc att ttt gat tct ttg gtt aaa aaa       288
Glu Met Thr Lys Lys Gln Val Phe Ile Phe Asp Ser Leu Val Lys Lys
                85                  90                  95

tgt tta ttt gaa gtg ctt aac aca aag aat ata ttt cct ggt gat gtt       336
Cys Leu Phe Glu Val Leu Asn Thr Lys Asn Ile Phe Pro Gly Asp Val
            100                 105                 110

aat tgg ttt gtg caa cat gaa tgg gga aaa gac caa ggc tgg cac tgc       384
Asn Trp Phe Val Gln His Glu Trp Gly Lys Asp Gln Gly Trp His Cys
        115                 120                 125

cat gta cta att gga gga aag gac ttt agt caa gct caa ggg aaa tgg       432
His Val Leu Ile Gly Gly Lys Asp Phe Ser Gln Ala Gln Gly Lys Trp
    130                 135                 140

tgg aga agg caa cta aat gtt tac tgg agc aga tgg ttg gta aca gcc       480
Trp Arg Arg Gln Leu Asn Val Tyr Trp Ser Arg Trp Leu Val Thr Ala
145                 150                 155                 160

tgt aat gtg caa cta aca cca gct gaa aga att aaa cta aga gaa ata       528
Cys Asn Val Gln Leu Thr Pro Ala Glu Arg Ile Lys Leu Arg Glu Ile
                165                 170                 175

gca gaa gac aat gag tgg gtt act cta ctt act tat aag cat aag caa       576
Ala Glu Asp Asn Glu Trp Val Thr Leu Leu Thr Tyr Lys His Lys Gln
            180                 185                 190

acc aaa aaa gac tat acc aag tgt gtt ctt ttt gga aac atg att gct       624
Thr Lys Lys Asp Tyr Thr Lys Cys Val Leu Phe Gly Asn Met Ile Ala
        195                 200                 205

tac tat ttt tta act aaa aag aaa ata agc act agt cca cca aga gac       672
Tyr Tyr Phe Leu Thr Lys Lys Lys Ile Ser Thr Ser Pro Pro Arg Asp
    210                 215                 220

gga ggc tat ttt ctt agc agt gac tct ggc tgg aaa act aac ttt tta       720
Gly Gly Tyr Phe Leu Ser Ser Asp Ser Gly Trp Lys Thr Asn Phe Leu
225                 230                 235                 240

aaa gaa ggc gag cgc cat cta gtg agc aaa cta tac act gat gac atg       768
Lys Glu Gly Glu Arg His Leu Val Ser Lys Leu Tyr Thr Asp Asp Met
                245                 250                 255

cgg cca gaa acg gtt gaa acc aca gta acc act gcg cag gaa act aag       816
Arg Pro Glu Thr Val Glu Thr Thr Val Thr Thr Ala Gln Glu Thr Lys
            260                 265                 270

cgc ggc aga att caa act aaa aaa gaa gtt tct att aaa act aca ctt       864
Arg Gly Arg Ile Gln Thr Lys Lys Glu Val Ser Ile Lys Thr Thr Leu
        275                 280                 285
```

```
aaa gag ctg gtg cat aaa aga gta acc tca cca gag gac tgg atg atg        912
Lys Glu Leu Val His Lys Arg Val Thr Ser Pro Glu Asp Trp Met Met
    290                 295                 300

atg cag cca gac agt tac att gaa atg atg gct caa cca ggt gga gaa        960
Met Gln Pro Asp Ser Tyr Ile Glu Met Met Ala Gln Pro Gly Gly Glu
305                 310                 315                 320

aac ctg ctg aaa aat acg cta gag att tgt aca cta act cta gcc aga       1008
Asn Leu Leu Lys Asn Thr Leu Glu Ile Cys Thr Leu Thr Leu Ala Arg
                325                 330                 335

acc aaa aca gca ttt gac tta att tta gaa aaa gct gaa acc agc aaa       1056
Thr Lys Thr Ala Phe Asp Leu Ile Leu Glu Lys Ala Glu Thr Ser Lys
            340                 345                 350

cta acc aac ttt tca ctg cct gac aca aga gcc tgc aga att ttt gct       1104
Leu Thr Asn Phe Ser Leu Pro Asp Thr Arg Ala Cys Arg Ile Phe Ala
        355                 360                 365

ttt cat ggc tgg aac tat gtt aaa gtt tgc cat gct att tgc tgt gtt       1152
Phe His Gly Trp Asn Tyr Val Lys Val Cys His Ala Ile Cys Cys Val
    370                 375                 380

tta aac aga caa gga ggc aaa aga aat act gtt tta ttt cat gga cca       1200
Leu Asn Arg Gln Gly Gly Lys Arg Asn Thr Val Leu Phe His Gly Pro
385                 390                 395                 400

gcc agc aca ggc aaa tct att att gca caa gcc ata gca caa gca gtt       1248
Ala Ser Thr Gly Lys Ser Ile Ile Ala Gln Ala Ile Ala Gln Ala Val
                405                 410                 415

ggc aat gtt ggt tgc tat aat gca gcc aat gta aac ttt cca ttt aat       1296
Gly Asn Val Gly Cys Tyr Asn Ala Ala Asn Val Asn Phe Pro Phe Asn
            420                 425                 430

gac tgt acc aac aag aac ttg att tgg gta gaa gaa gct ggt aac ttt       1344
Asp Cys Thr Asn Lys Asn Leu Ile Trp Val Glu Glu Ala Gly Asn Phe
        435                 440                 445

gga cag caa gta aac cag ttt aaa gcc att tgc tct ggt caa act att       1392
Gly Gln Gln Val Asn Gln Phe Lys Ala Ile Cys Ser Gly Gln Thr Ile
    450                 455                 460

cgc att gat caa aaa gga aaa ggc agc aaa cag att gaa cca aca cca       1440
Arg Ile Asp Gln Lys Gly Lys Gly Ser Lys Gln Ile Glu Pro Thr Pro
465                 470                 475                 480

gtc atc atg acc aca aat gag aac att aca gtg gtc aga ata ggc tgc       1488
Val Ile Met Thr Thr Asn Glu Asn Ile Thr Val Val Arg Ile Gly Cys
                485                 490                 495

gaa gaa aga cca gaa cac act caa cca atc aga gac aga atg ctt aac       1536
Glu Glu Arg Pro Glu His Thr Gln Pro Ile Arg Asp Arg Met Leu Asn
            500                 505                 510

att cat cta aca cat acc ttg cct ggt gac ttt ggt ttg gtt gac aaa       1584
Ile His Leu Thr His Thr Leu Pro Gly Asp Phe Gly Leu Val Asp Lys
        515                 520                 525

aat gaa tgg ccc atg att tgt gct tgg ttg gta aag aat ggt tac caa       1632
Asn Glu Trp Pro Met Ile Cys Ala Trp Leu Val Lys Asn Gly Tyr Gln
    530                 535                 540

tct acc atg gca agc tac tgt gct aaa tgg ggc aaa gtt cct gat tgg       1680
Ser Thr Met Ala Ser Tyr Cys Ala Lys Trp Gly Lys Val Pro Asp Trp
545                 550                 555                 560
```

```
tca gaa aac tgg gcg gag cca aag gtg cca act cct ata aat tta cta      1728
Ser Glu Asn Trp Ala Glu Pro Lys Val Pro Thr Pro Ile Asn Leu Leu
                565                 570                 575

ggt tcg gca cgc tca cca ttc acg aca ccg aaa agt acg cct ctc agc      1776
Gly Ser Ala Arg Ser Pro Phe Thr Thr Pro Lys Ser Thr Pro Leu Ser
            580                 585                 590

cag aac tat gca cta act cca ctt gca tcg gat ctc gag gac ctg gct      1824
Gln Asn Tyr Ala Leu Thr Pro Leu Ala Ser Asp Leu Glu Asp Leu Ala
        595                 600                 605

tta gag cct tgg agc aca cca aat act cct gtt gcg ggc act gca gaa      1872
Leu Glu Pro Trp Ser Thr Pro Asn Thr Pro Val Ala Gly Thr Ala Glu
    610                 615                 620

acc cag aac act ggg gaa gct ggt tcc aaa gcc tgc caa gat ggt caa      1920
Thr Gln Asn Thr Gly Glu Ala Gly Ser Lys Ala Cys Gln Asp Gly Gln
625                 630                 635                 640

ctg agc cca act tgg tca gag atc gag gag gat ttg aga gcg tgc ttc      1968
Leu Ser Pro Thr Trp Ser Glu Ile Glu Glu Asp Leu Arg Ala Cys Phe
                645                 650                 655

ggt gcg gaa ccg ttg aag aaa gac ttc agc gag ccg ctg aac ttg gac      2016
Gly Ala Glu Pro Leu Lys Lys Asp Phe Ser Glu Pro Leu Asn Leu Asp
            660                 665                 670

taa                                                                 2019
```

<210> 9
<211> 20
<212> PRT
<213> part of Parvovirus NS1 variant

<400> 9

```
Thr Arg Ala Cys Arg Ile Phe Ala Phe His Gly Trp Asn Tyr Val Lys
1               5                   10                  15

Val Cys His Ala
            20
```

<210> 10
<211> 672
<212> PRT
<213> Parvovirus NS1 variant

<400> 10

```
Met Ala Gly Asn Ala Tyr Ser Asp Glu Val Leu Gly Ala Thr Asn Trp
  1               5                  10                  15

Leu Lys Glu Lys Ser Asn Gln Glu Val Phe Ser Phe Val Phe Lys Asn
            20                  25                  30

Glu Asn Val Gln Leu Asn Gly Lys Asp Ile Gly Trp Asn Ser Tyr Lys
        35                  40                  45

Lys Glu Leu Gln Glu Asp Glu Leu Lys Ser Leu Gln Arg Gly Ala Glu
    50                  55                  60
```

```
Thr Thr Trp Asp Gln Ser Glu Asp Met Glu Trp Glu Thr Thr Val Asp
 65                  70                  75                  80

Glu Met Thr Lys Lys Gln Val Phe Ile Phe Asp Ser Leu Val Lys Lys
                85                  90                  95

Cys Leu Phe Glu Val Leu Asn Thr Lys Asn Ile Phe Pro Gly Asp Val
            100                 105                 110

Asn Trp Phe Val Gln His Glu Trp Gly Lys Asp Gln Gly Trp His Cys
        115                 120                 125

His Val Leu Ile Gly Gly Lys Asp Phe Ser Gln Ala Gln Gly Lys Trp
    130                 135                 140

Trp Arg Arg Gln Leu Asn Val Tyr Trp Ser Arg Trp Leu Val Thr Ala
145                 150                 155                 160

Cys Asn Val Gln Leu Thr Pro Ala Glu Arg Ile Lys Leu Arg Glu Ile
                165                 170                 175

Ala Glu Asp Asn Glu Trp Val Thr Leu Leu Thr Tyr Lys His Lys Gln
            180                 185                 190

Thr Lys Lys Asp Tyr Thr Lys Cys Val Leu Phe Gly Asn Met Ile Ala
        195                 200                 205

Tyr Tyr Phe Leu Thr Lys Lys Lys Ile Ser Thr Ser Pro Pro Arg Asp
    210                 215                 220

Gly Gly Tyr Phe Leu Ser Ser Asp Ser Gly Trp Lys Thr Asn Phe Leu
225                 230                 235                 240

Lys Glu Gly Glu Arg His Leu Val Ser Lys Leu Tyr Thr Asp Asp Met
                245                 250                 255

Arg Pro Glu Thr Val Glu Thr Thr Val Thr Thr Ala Gln Glu Thr Lys
            260                 265                 270

Arg Gly Arg Ile Gln Thr Lys Lys Glu Val Ser Ile Lys Thr Thr Leu
        275                 280                 285

Lys Glu Leu Val His Lys Arg Val Thr Ser Pro Glu Asp Trp Met Met
    290                 295                 300

Met Gln Pro Asp Ser Tyr Ile Glu Met Met Ala Gln Pro Gly Gly Glu
305                 310                 315                 320

Asn Leu Leu Lys Asn Thr Leu Glu Ile Cys Thr Leu Thr Leu Ala Arg
                325                 330                 335

Thr Lys Thr Ala Phe Asp Leu Ile Leu Glu Lys Ala Glu Thr Ser Lys
            340                 345                 350

Leu Thr Asn Phe Ser Leu Pro Asp Thr Arg Ala Cys Arg Ile Phe Ala
        355                 360                 365

Phe His Gly Trp Asn Tyr Val Lys Val Cys His Ala Ile Cys Cys Val
    370                 375                 380

Leu Asn Arg Gln Gly Gly Lys Arg Asn Thr Val Leu Phe His Gly Pro
385                 390                 395                 400

Ala Ser Thr Gly Lys Ser Ile Ile Ala Gln Ala Ile Ala Gln Ala Val
                405                 410                 415
```

Gly Asn Val Gly Cys Tyr Asn Ala Ala Asn Val Asn Phe Pro Phe Asn
420 425 430

Asp Cys Thr Asn Lys Asn Leu Ile Trp Val Glu Glu Ala Gly Asn Phe
435 440 445

Gly Gln Gln Val Asn Gln Phe Lys Ala Ile Cys Ser Gly Gln Thr Ile
450 455 460

Arg Ile Asp Gln Lys Gly Lys Gly Ser Lys Gln Ile Glu Pro Thr Pro
465 470 475 480

Val Ile Met Thr Thr Asn Glu Asn Ile Thr Val Val Arg Ile Gly Cys
485 490 495

Glu Glu Arg Pro Glu His Thr Gln Pro Ile Arg Asp Arg Met Leu Asn
500 505 510

Ile His Leu Thr His Thr Leu Pro Gly Asp Phe Gly Leu Val Asp Lys
515 520 525

Asn Glu Trp Pro Met Ile Cys Ala Trp Leu Val Lys Asn Gly Tyr Gln
530 535 540

Ser Thr Met Ala Ser Tyr Cys Ala Lys Trp Gly Lys Val Pro Asp Trp
545 550 555 560

Ser Glu Asn Trp Ala Glu Pro Lys Val Pro Thr Pro Ile Asn Leu Leu
565 570 575

Gly Ser Ala Arg Ser Pro Phe Thr Thr Pro Lys Ser Thr Pro Leu Ser
580 585 590

Gln Asn Tyr Ala Leu Thr Pro Leu Ala Ser Asp Leu Glu Asp Leu Ala
595 600 605

Leu Glu Pro Trp Ser Thr Pro Asn Thr Pro Val Ala Gly Thr Ala Glu
610 615 620

Thr Gln Asn Thr Gly Glu Ala Gly Ser Lys Ala Cys Gln Asp Gly Gln
625 630 635 640

Leu Ser Pro Thr Trp Ser Glu Ile Glu Glu Asp Leu Arg Ala Cys Phe
645 650 655

Gly Ala Glu Pro Leu Lys Lys Asp Phe Ser Glu Pro Leu Asn Leu Asp
660 665 670

<210> 11
<211> 60
<212> DNA
<213> part of Parvovirus NS1 variant

<220>
<221> CDS
<222> (1)..(60)

<400> 11

att tgc tgt gtt tta aac aga caa gga ggc aaa aga aat gct gtt tta 48
Ile Cys Cys Val Leu Asn Arg Gln Gly Gly Lys Arg Asn Ala Val Leu
1 5 10 15

ttt cat gga cca 60
Phe His Gly Pro
20

<210> 12
<211> 2019
<212> DNA
<213> Parvovirus NS1 variant

<220>
<221> CDS
<222> (1)..(2016)

<400> 12

atg gct gga aat gct tac tct gat gaa gtt ttg gga gca acc aac tgg 48
Met Ala Gly Asn Ala Tyr Ser Asp Glu Val Leu Gly Ala Thr Asn Trp
1 5 10 15

tta aag gaa aaa agt aac cag gaa gtg ttc tca ttt gtt ttt aaa aat 96
Leu Lys Glu Lys Ser Asn Gln Glu Val Phe Ser Phe Val Phe Lys Asn
20 25 30

gaa aat gtt caa ctg aat gga aaa gat atc gga tgg aat agt tac aaa 144
Glu Asn Val Gln Leu Asn Gly Lys Asp Ile Gly Trp Asn Ser Tyr Lys
35 40 45

aaa gag ctg cag gag gac gag ctg aaa tct tta caa cga gga gcg gaa 192
Lys Glu Leu Gln Glu Asp Glu Leu Lys Ser Leu Gln Arg Gly Ala Glu
50 55 60

act act tgg gac caa agc gag gac atg gaa tgg gaa acc aca gtg gat 240
Thr Thr Trp Asp Gln Ser Glu Asp Met Glu Trp Glu Thr Thr Val Asp
65 70 75 80

gaa atg acc aaa aag caa gta ttc att ttt gat tct ttg gtt aaa aaa 288
Glu Met Thr Lys Lys Gln Val Phe Ile Phe Asp Ser Leu Val Lys Lys
85 90 95

tgt tta ttt gaa gtg ctt aac aca aag aat ata ttt cct ggt gat gtt 336
Cys Leu Phe Glu Val Leu Asn Thr Lys Asn Ile Phe Pro Gly Asp Val
100 105 110

aat tgg ttt gtg caa cat gaa tgg gga aaa gac caa ggc tgg cac tgc 384
Asn Trp Phe Val Gln His Glu Trp Gly Lys Asp Gln Gly Trp His Cys
115 120 125

cat gta cta att gga gga aag gac ttt agt caa gct caa ggg aaa tgg 432
His Val Leu Ile Gly Gly Lys Asp Phe Ser Gln Ala Gln Gly Lys Trp
130 135 140

tgg aga agg caa cta aat gtt tac tgg agc aga tgg ttg gta aca gcc 480
Trp Arg Arg Gln Leu Asn Val Tyr Trp Ser Arg Trp Leu Val Thr Ala
145 150 155 160

tgt aat gtg caa cta aca cca gct gaa aga att aaa cta aga gaa ata 528
Cys Asn Val Gln Leu Thr Pro Ala Glu Arg Ile Lys Leu Arg Glu Ile
165 170 175

gca gaa gac aat gag tgg gtt act cta ctt act tat aag cat aag caa 576
Ala Glu Asp Asn Glu Trp Val Thr Leu Leu Thr Tyr Lys His Lys Gln
180 185 190

```
acc aaa aaa gac tat acc aag tgt gtt ctt ttt gga aac atg att gct      624
Thr Lys Lys Asp Tyr Thr Lys Cys Val Leu Phe Gly Asn Met Ile Ala
        195                 200                 205

tac tat ttt tta act aaa aag aaa ata agc act agt cca cca aga gac      672
Tyr Tyr Phe Leu Thr Lys Lys Lys Ile Ser Thr Ser Pro Pro Arg Asp
    210                 215                 220

gga ggc tat ttt ctt agc agt gac tct ggc tgg aaa act aac ttt tta      720
Gly Gly Tyr Phe Leu Ser Ser Asp Ser Gly Trp Lys Thr Asn Phe Leu
225                 230                 235                 240

aaa gaa ggc gag cgc cat cta gtg agc aaa cta tac act gat gac atg      768
Lys Glu Gly Glu Arg His Leu Val Ser Lys Leu Tyr Thr Asp Asp Met
                245                 250                 255

cgg cca gaa acg gtt gaa acc aca gta acc act gcg cag gaa act aag      816
Arg Pro Glu Thr Val Glu Thr Thr Val Thr Thr Ala Gln Glu Thr Lys
            260                 265                 270

cgc ggc aga att caa act aaa aaa gaa gtt tct att aaa act aca ctt      864
Arg Gly Arg Ile Gln Thr Lys Lys Glu Val Ser Ile Lys Thr Thr Leu
        275                 280                 285

aaa gag ctg gtg cat aaa aga gta acc tca cca gag gac tgg atg atg      912
Lys Glu Leu Val His Lys Arg Val Thr Ser Pro Glu Asp Trp Met Met
    290                 295                 300

atg cag cca gac agt tac att gaa atg atg gct caa cca ggt gga gaa      960
Met Gln Pro Asp Ser Tyr Ile Glu Met Met Ala Gln Pro Gly Gly Glu
305                 310                 315                 320

aac ctg ctg aaa aat acg cta gag att tgt aca cta act cta gcc aga     1008
Asn Leu Leu Lys Asn Thr Leu Glu Ile Cys Thr Leu Thr Leu Ala Arg
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acc aaa aca gca ttt gac tta att tta gaa aaa gct gaa acc agc aaa     1056
Thr Lys Thr Ala Phe Asp Leu Ile Leu Glu Lys Ala Glu Thr Ser Lys
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cta acc aac ttt tca ctg cct gac aca aga acc tgc aga att ttt gct     1104
Leu Thr Asn Phe Ser Leu Pro Asp Thr Arg Thr Cys Arg Ile Phe Ala
        355                 360                 365

ttt cat ggc tgg aac tat gtt aaa gtt tgc cat gct att tgc tgt gtt     1152
Phe His Gly Trp Asn Tyr Val Lys Val Cys His Ala Ile Cys Cys Val
    370                 375                 380

tta aac aga caa gga ggc aaa aga aat gct gtt tta ttt cat gga cca     1200
Leu Asn Arg Gln Gly Gly Lys Arg Asn Ala Val Leu Phe His Gly Pro
385                 390                 395                 400

gcc agc aca ggc aaa tct att att gca caa gcc ata gca caa gca gtt     1248
Ala Ser Thr Gly Lys Ser Ile Ile Ala Gln Ala Ile Ala Gln Ala Val
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ggc aat gtt ggt tgc tat aat gca gcc aat gta aac ttt cca ttt aat     1296
Gly Asn Val Gly Cys Tyr Asn Ala Ala Asn Val Asn Phe Pro Phe Asn
            420                 425                 430

gac tgt acc aac aag aac ttg att tgg gta gaa gaa gct ggt aac ttt     1344
Asp Cys Thr Asn Lys Asn Leu Ile Trp Val Glu Glu Ala Gly Asn Phe
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gtc atc atg acc aca aat gag aac att aca gtg gtc aga ata ggc tgc      1488
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aat gaa tgg ccc atg att tgt gct tgg ttg gta aag aat ggt tac caa      1632
Asn Glu Trp Pro Met Ile Cys Ala Trp Leu Val Lys Asn Gly Tyr Gln
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tct acc atg gca agc tac tgt gct aaa tgg ggc aaa gtt cct gat tgg      1680
Ser Thr Met Ala Ser Tyr Cys Ala Lys Trp Gly Lys Val Pro Asp Trp
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tca gaa aac tgg gcg gag cca aag gtg cca act cct ata aat tta cta      1728
Ser Glu Asn Trp Ala Glu Pro Lys Val Pro Thr Pro Ile Asn Leu Leu
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ggt tcg gca cgc tca cca ttc acg aca ccg aaa agt acg cct ctc agc      1776
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            580                 585                 590

cag aac tat gca cta act cca ctt gca tcg gat ctc gag gac ctg gct      1824
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        595                 600                 605

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Leu Glu Pro Trp Ser Thr Pro Asn Thr Pro Val Ala Gly Thr Ala Glu
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acc cag aac act ggg gaa gct ggt tcc aaa gcc tgc caa gat ggt caa      1920
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625                 630                 635                 640

ctg agc cca act tgg tca gag atc gag gag gat ttg aga gcg tgc ttc      1968
Leu Ser Pro Thr Trp Ser Glu Ile Glu Glu Asp Leu Arg Ala Cys Phe
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ggt gcg gaa ccg ttg aag aaa gac ttc agc gag ccg ctg aac ttg gac      2016
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<210> 14
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245 250 255

Arg Pro Glu Thr Val Glu Thr Thr Val Thr Thr Ala Gln Glu Thr Lys
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Arg Gly Arg Ile Gln Thr Lys Lys Glu Val Ser Ile Lys Thr Thr Leu
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Phe His Gly Trp Asn Tyr Val Lys Val Cys His Ala Ile Cys Cys Val
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Ala Ser Thr Gly Lys Ser Ile Ile Ala Gln Ala Ile Ala Gln Ala Val
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Arg Ile Asp Gln Lys Gly Lys Gly Ser Lys Gln Ile Glu Pro Thr Pro
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tta aag gaa aaa agt aac cag gaa gtg ttc tca ttt gtt ttt aaa aat 96
Leu Lys Glu Lys Ser Asn Gln Glu Val Phe Ser Phe Val Phe Lys Asn
20 25 30

gaa aat gtt caa ctg aat gga aaa gat atc gga tgg aat agt tac aaa 144
Glu Asn Val Gln Leu Asn Gly Lys Asp Ile Gly Trp Asn Ser Tyr Lys
35 40 45

aaa gag ctg cag gag gac gag ctg aaa tct tta caa cga gga gcg gaa 192
Lys Glu Leu Gln Glu Asp Glu Leu Lys Ser Leu Gln Arg Gly Ala Glu
50 55 60

act act tgg gac caa agc gag gac atg gaa tgg gaa acc aca gtg gat 240
Thr Thr Trp Asp Gln Ser Glu Asp Met Glu Trp Glu Thr Thr Val Asp
65 70 75 80

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gaa atg acc aaa aag caa gta ttc att ttt gat tct ttg gtt aaa aaa        288
Glu Met Thr Lys Lys Gln Val Phe Ile Phe Asp Ser Leu Val Lys Lys
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tgt tta ttt gaa gtg ctt aac aca aag aat ata ttt cct ggt gat gtt        336
Cys Leu Phe Glu Val Leu Asn Thr Lys Asn Ile Phe Pro Gly Asp Val
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aat tgg ttt gtg caa cat gaa tgg gga aaa gac caa ggc tgg cac tgc        384
Asn Trp Phe Val Gln His Glu Trp Gly Lys Asp Gln Gly Trp His Cys
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cat gta cta att gga gga aag gac ttt agt caa gct caa ggg aaa tgg        432
His Val Leu Ile Gly Gly Lys Asp Phe Ser Gln Ala Gln Gly Lys Trp
    130                 135                 140

tgg aga agg caa cta aat gtt tac tgg agc aga tgg ttg gta aca gcc        480
Trp Arg Arg Gln Leu Asn Val Tyr Trp Ser Arg Trp Leu Val Thr Ala
145                 150                 155                 160

tgt aat gtg caa cta aca cca gct gaa aga att aaa cta aga gaa ata        528
Cys Asn Val Gln Leu Thr Pro Ala Glu Arg Ile Lys Leu Arg Glu Ile
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gca gaa gac aat gag tgg gtt act cta ctt act tat aag cat aag caa        576
Ala Glu Asp Asn Glu Trp Val Thr Leu Leu Thr Tyr Lys His Lys Gln
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acc aaa aaa gac tat acc aag tgt gtt ctt ttt gga aac atg att gct        624
Thr Lys Lys Asp Tyr Thr Lys Cys Val Leu Phe Gly Asn Met Ile Ala
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tac tat ttt tta act aaa aag aaa ata agc act agt cca cca aga gac        672
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gga ggc tat ttt ctt agc agt gac tct ggc tgg aaa act aac ttt tta        720
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aaa gaa ggc gag cgc cat cta gtg agc aaa cta tac act gat gac atg        768
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cgg cca gaa acg gtt gaa acc aca gta acc act gcg cag gaa act aag        816
Arg Pro Glu Thr Val Glu Thr Thr Val Thr Thr Ala Gln Glu Thr Lys
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cgc ggc aga att caa act aaa aaa gaa gtt tct att aaa act aca ctt        864
Arg Gly Arg Ile Gln Thr Lys Lys Glu Val Ser Ile Lys Thr Thr Leu
        275                 280                 285

aaa gag ctg gtg cat aaa aga gta acc tca cca gag gac tgg atg atg        912
Lys Glu Leu Val His Lys Arg Val Thr Ser Pro Glu Asp Trp Met Met
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atg cag cca gac agt tac att gaa atg atg gct caa cca ggt gga gaa        960
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305                 310                 315                 320

aac ctg ctg aaa aat acg cta gag att tgt aca cta act cta gcc aga       1008
Asn Leu Leu Lys Asn Thr Leu Glu Ile Cys Thr Leu Thr Leu Ala Arg
                325                 330                 335

acc aaa aca gca ttt gac tta att tta gaa aaa gct gaa acc agc aaa       1056
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ttt cat ggc tgg aac tat gtt aaa gtt tgc cat gct att tgc tgt gtt      1152
Phe His Gly Trp Asn Tyr Val Lys Val Cys His Ala Ile Cys Cys Val
    370                 375                 380

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ggc aat gtt ggt tgc tat aat gca gcc aat gta aac ttt cca ttt aat      1296
Gly Asn Val Gly Cys Tyr Asn Ala Ala Asn Val Asn Phe Pro Phe Asn
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gac tgt acc aac aag aac ttg att tgg gta gaa gaa gct ggt aac ttt      1344
Asp Cys Thr Asn Lys Asn Leu Ile Trp Val Glu Glu Ala Gly Asn Phe
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gga cag caa gta aac cag ttt aaa gcc att tgc tct ggt caa gct att      1392
Gly Gln Gln Val Asn Gln Phe Lys Ala Ile Cys Ser Gly Gln Ala Ile
    450                 455                 460

cgc att gat caa aaa gga aaa ggc agc aaa cag att gaa cca aca cca      1440
Arg Ile Asp Gln Lys Gly Lys Gly Ser Lys Gln Ile Glu Pro Thr Pro
465                 470                 475                 480

gtc atc atg acc aca aat gag aac att aca gtg gtc aga ata ggc tgc      1488
Val Ile Met Thr Thr Asn Glu Asn Ile Thr Val Val Arg Ile Gly Cys
                485                 490                 495

gaa gaa aga cca gaa cac act caa cca atc aga gac aga atg ctt aac      1536
Glu Glu Arg Pro Glu His Thr Gln Pro Ile Arg Asp Arg Met Leu Asn
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Ile His Leu Thr His Thr Leu Pro Gly Asp Phe Gly Leu Val Asp Lys
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Gly Ser Ala Arg Ser Pro Phe Thr Thr Pro Lys Ser Thr Pro Leu Ser
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cag aac tat gca cta act cca ctt gca tcg gat ctc gag gac ctg gct      1824
Gln Asn Tyr Ala Leu Thr Pro Leu Ala Ser Asp Leu Glu Asp Leu Ala
        595                 600                 605

tta gag cct tgg agc aca cca aat act cct gtt gcg ggc act gca gaa      1872
Leu Glu Pro Trp Ser Thr Pro Asn Thr Pro Val Ala Gly Thr Ala Glu
    610                 615                 620
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ctg agc cca act tgg tca gag atc gag gag gat ttg aga gcg tgc ttc      1968
Leu Ser Pro Thr Trp Ser Glu Ile Glu Glu Asp Leu Arg Ala Cys Phe
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ggt gcg gaa ccg ttg aag aaa gac ttc agc gag ccg ctg aac ttg gac      2016
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taa                                                                 2019
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<210> 17
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Lys Glu Leu Gln Glu Asp Glu Leu Lys Ser Leu Gln Arg Gly Ala Glu
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Thr Thr Trp Asp Gln Ser Glu Asp Met Glu Trp Glu Thr Thr Val Asp
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Glu Met Thr Lys Lys Gln Val Phe Ile Phe Asp Ser Leu Val Lys Lys
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Cys Leu Phe Glu Val Leu Asn Thr Lys Asn Ile Phe Pro Gly Asp Val
            100                 105                 110

Asn Trp Phe Val Gln His Glu Trp Gly Lys Asp Gln Gly Trp His Cys
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His Val Leu Ile Gly Gly Lys Asp Phe Ser Gln Ala Gln Gly Lys Trp
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Cys Asn Val Gln Leu Thr Pro Ala Glu Arg Ile Lys Leu Arg Glu Ile
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195 200 205

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Gly Gly Tyr Phe Leu Ser Ser Asp Ser Gly Trp Lys Thr Asn Phe Leu
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Lys Glu Gly Glu Arg His Leu Val Ser Lys Leu Tyr Thr Asp Asp Met
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340 345 350

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Phe His Gly Trp Asn Tyr Val Lys Val Cys His Ala Ile Cys Cys Val
370 375 380

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Ala Ser Thr Gly Lys Ser Ile Ile Ala Gln Ala Ile Ala Gln Ala Val
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Asp Cys Thr Asn Lys Asn Leu Ile Trp Val Glu Glu Ala Gly Asn Phe
435 440 445

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Arg Ile Asp Gln Lys Gly Lys Gly Ser Lys Gln Ile Glu Pro Thr Pro
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500 505 510

Ile His Leu Thr His Thr Leu Pro Gly Asp Phe Gly Leu Val Asp Lys
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Asn Glu Trp Pro Met Ile Cys Ala Trp Leu Val Lys Asn Gly Tyr Gln
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Ser Thr Met Ala Ser Tyr Cys Ala Lys Trp Gly Lys Val Pro Asp Trp
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565 570 575

Gly Ser Ala Arg Ser Pro Phe Thr Thr Pro Lys Ser Thr Pro Leu Ser
580 585 590

Gln Asn Tyr Ala Leu Thr Pro Leu Ala Ser Asp Leu Glu Asp Leu Ala
595 600 605

Leu Glu Pro Trp Ser Thr Pro Asn Thr Pro Val Ala Gly Thr Ala Glu
610 615 620

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625 630 635 640

Leu Ser Pro Thr Trp Ser Glu Ile Glu Glu Asp Leu Arg Ala Cys Phe
645 650 655

Gly Ala Glu Pro Leu Lys Lys Asp Phe Ser Glu Pro Leu Asn Leu Asp
660 665 670

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/07835

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/35 C07K14/015 C07K16/08 G01N33/569 C12Q1/70
A61K35/76 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, STRAND, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	D LEGENDRE & J ROMMELAERE: "Terminal regions of the NS-1 protein of the parvovirus Minute Virus of Mice are involved in cytotoxicity and promoter trans inhibition" JOURNAL OF VIROLOGY, vol. 66, no. 10, October 1992 (1992-10), pages 5705-5713, XP000867510 AMERICAN SOCIETY FOR MICROBIOLOGY US *mutants pMMBal31 and pULB3201; figure 1 and page 5710 first paragraph* --- -/--	1-5,7-11

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 January 2001	30.01.01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Cupido, M

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	LI X ET AL: "Mutation of lysine 405 to serine in the parvovirus H-1 NS1 abolishes its function for viral DNA replication, late promoter activation, and cytotoxocity" JOURNAL OF VIROLOGY, vol. 64, no. 10, October 1990 (1990-10), pages 4654-4660, XP000867496 AMERICAN SOCIETY FOR MICROBIOLOGY US page 4656 -page 4657 ---	1,3,7, 9-11
A	J P F NÜESCH ET AL: "Sequence motifs in the replicator protein of parvovirus MVM essential for nicking and covalent attachement to the viral origin: identification of the linking tyrosine" VIROLOGY,US,ACADEMIC PRESS,ORLANDO, vol. 209, no. 1, 10 May 1995 (1995-05-10), pages 122-135, XP002088311 ISSN: 0042-6822 page 127 -page 131 ---	1-13
A	S F COTTMORE ET AL: "The NS1 polypeptide of the murine parvovirus MVM binds to DNA sequences containing the motif (ACCA)2-3" JOURNAL OF VIROLOGY,US,THE AMERICAN SOCIETY FOR MICROBIOLOGY, vol. 69, no. 3, pages 1652-1660, XP002088309 ISSN: 0022-538X page 1658, left-hand column, last paragraph -right-hand column -----	1-13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 00/07835

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 14 and 15 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the NS1 variant.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.